

Screening the antifungal properties of essential oils against *Phytophthora colocasiae*

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Research Article

Keywords: Biocontrol, Phytochemicals, *Phytophthora colocasiae*, Sage and tea tree essential oil

Posted Date: December 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1042822/v1>

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Abstract

Recently Microbicidal and micro-static activities of naturally obtained botanicals have been extensively explored, generally in response to the devastating apprehension of consumers towards the safety of edible products. However, scientists are paying more interest in using bio pesticides and phytochemicals that constitute environmentally favorable, non-toxic, long-lasting, and productive substitutes for preventing many hazardous plant pathogens. This research aimed to investigate the antifungal capabilities of sage and tea tree essential oils towards taro leaf blight, the most important cause of worldwide production losses. Using synthetic fungicides is a rapid and effective approach for controlling plant diseases, but it also creates human health risks, environmental threats, and chances of pathogen resistance. The essential oils of sage and tea tree were obtained by microwave-assisted hydro-distillation, and their chemical components were analyzed using FTIR spectroscopy. The main components of the oil were Thujone and Terpinen-4-ol, in sage and tea, respectively. A casual disease agent isolated from symptomatic taro leaves is used as a test fungus and identified as *Phytophthora colocasiae*. The antifungal properties of both essential oils were evaluated against mycelium, sporangium, zoospores, leaf necrosis, and corm lesions. Repeated experiments showed that the minimum concentrations for obtaining 100% inhibition of mycelium, zoospore germination, sporangia formation, and leaf necrosis were estimated at 2.5 and 5.0 mg/mL of sage and tea tree oils, respectively. Outcomes of this study provide an allusion for the prevention and curation of plant diseases promptly, economically, and environmentally by using phytochemicals and plant essential oil derivatives.

1. Introduction

Essential oils (EO), also known as volatiles, ethereal oils, or essences, are biochemical chemicals produced by several different species, most probably plants (Vasconcelos et al., 2019). These oils are commonly obtained using classical approaches, including distillation, pressurizing, or solvent-based extraction (Ali, Chua, & Chow, 2019). Because of their nature and characteristics, EOs are extensively used in scents, foodstuffs, skincare, and pharmacology (Szutt, Dołhańczuk-Śródka, & Sporek, 2019). The biochemical makeup of various plant oils varies depending on wherever they grow and particularly on the climatic conditions (Arraiza, 2017). Nevertheless, the essential oil composition may change with the methods employed for drying plant materials, extraction, and distilling process (Merah et al., 2020). These oils are valued for their pleasant fragrance and bioactive components and characteristics (Liao et al., 2017). Essential oils are typically extracted via conventional hydro-distillation (Elyemni et al., 2019), which decreases oil quality and exhibits minimal extraction efficiency when certain volatile compounds can be lost during the extraction and the deprivation of unsaturated compositions temperatures fluctuations and nature of solvents used (Chen et al., 2020). Microwave-assisted extraction, a unique and inventive methodology, has received considerable research and is suitable for small materials and simple and effective control overheated mechanisms (Vinatoru, Mason, & Calinescu, 2017). The primary advantages of microwave-assisted extracting are affordable, time-saving, eco-friendly, and solvent-free extraction (Y. Wang et al., 2018).

Taro *Colocasia esculenta* L. is a well-known tuber crop that is extensively grown in many regions of the world, especially China, Africa, and European countries, consumed as a staple food (Ahmed et al., 2020). Taro corms and leaves are rich in carbohydrates, proteins, minerals, and vitamins (Sharma, Jan, Kaur, & Riar, 2020), and essential elements, including calcium, phosphorus, iron, vitamin C, thiamine, riboflavin, and niacin (Otieno,

2020). Furthermore, taro has medicinal potentials against several pathogenic diseases and physiological disorders in humans. Taro is vulnerable to over 23 various diseases that negatively impact plant development and production. Taro leaf blight is the most significant damaging of these diseases, resulting in reductions in good crop production (M. E. Bi, Teke, Christopher, Annih, & Charles, 2020). This disease is very common and has resulted in significant production losses for the crop in different cultivation areas of China (Z. Wang et al., 2021). *Phytophthora colocasiae* predominantly infects foliar parts of plants, but in severe cases can also appear on petioles and damage the corms of the hosts. The disease develops around 2 to 4 days following infection, during warmer humid climates, and is particularly noticeable on the top portion of plant leaves.

The initial symptoms appear as small, light, or dark brown specks, mainly at the lamina and margins of leaves where water accrues. The patches expand rapidly, fetching spherical, zonate, and purple-brown colors. A watery substance oozes out underneath the spots and appears as a dry gray and solid bubbles like plant exudate. (Jeeva, Veena, Makesh Kumar, & Arutselvan, 2020). In favorable conditions, the disease becomes severe, the lesions become dark brown with yellow edges, and an ash-colored ring of sporangia at the borders of the lesions becomes apparent. Lesions turn papery and drop off under dry and hot environments, causing shot-holes appearance in leaves. However, grown taro leaves become destroyed in few days, dangling like flags and possibly falling on the ground whenever petioles get damaged. The disease causes 50-60% losses, leading to significant corms deterioration immediately or after harvesting (Takor, Monono, Ntane, Ngale, & Fontem, 2020).

Phytophthora colocasiae is an oomycete fungus and has a complicated lifecycle depending on growth habits, including hemibiotrophic, biotrophic, and necrotrophic (Alexandra, Jamora, Smale, & Ghanem, 2020). The survival temperature for pathogen ranges between 15-35 °C. The optimum temperature from 25-30 °C when the night temperature is around 20 °C, and the moisture content exceeds 90%, encourages zoospore to germinate rapidly, triggering disease outbreaks. *P. colocasiae* is a heterothallic species that produce asexual sporangia, sporangiophores, zoospores, and sexually oospores that become vested in plant debris and corms inoculum for upcoming cropping season. The pathogen overwinters by forming chlamydospores through conversion of thick-walled zoospores, hibernating for persistent viability in the plant debris and corms (Brooks, 2015). Such process enables *P. colocasiae*, as a dangerous pathogen, while continual farming harbors pathogen in field biomass continuously persist in the soil.

The disease can be cured effectively with systemic fungicides, but on the other hand, it creates an immediate risk to human and environmental health as well as long-term consequences on biodiversity and the ecosystem. People are increasingly opting for chemical-free products, particularly those produced in organic agriculture. Thus, researchers focus on bio-pesticides and phytochemicals as eco-friendly, non-hazardous, long-lasting, and effective alternatives to manage plant diseases.

Essential oils or combinations of different phytochemicals exhibit synergistic potential with additional properties against many plant pathogens. This advocates a cost-effective and natural alternative to both agro-food production and consumers, at the same while to the obstacles in innovations to inhibit proliferation of plant pathogens. Sage (*salvia sclarea*) and tea tree (*Melaleuca alternifolia*) essential oils contain high antibacterial activities. They are being used as a natural cure and preventive for many plant diseases (Chidi, Bouhoudan, & Khaddor, 2020; Ebani et al., 2018). As far as our knowledge is concerned, there is no prior

information for using essential oil from sage and tea tree against taro leaf blight. The present study was designed to explore and develop an easy, compact and effective system for extracting essential oils for convenient and consistent control of plant diseases for sustainable food production.

2. Materials And Methods

Freshly collected Sage and tea tree plant materials (leaves, stems) were thoroughly rinsed in sterile water and dried in ventilation under shade at room temperature. Plant parts were crushed into small pieces and soaked in sterilized distilled water overnight before extraction.

2.1 Extraction of essential oil

The essential oils of sage and tea trees were obtained using an especially designed microwave-assisted hydro-distillation (MAHD) machine mounted with a Clevenger apparatus. The machine was modified with a swift temperature controller for initial and final heating requirements for different types of plant materials. The idea behind this machine is that microwave breaks the cell wall of plant cells and ruptures the cell and swift release of oil components. It operates with a microwave power of 800Watt; rated input wattage; 1000 Watt: operates at a frequency of about 2.4 GHz; Maximum Voltage 120 volts AC.; maximum temp: 300°C. The equipment has a digital control panel, a touch LCD, and an automated program to extract different plant oils at different times and temperature requirements. The capacity of the extraction container was 5Litre and connected to Clevenger with rubber pipes for water circulation. The main advantages of this machine include compact size, need small space, stirring device to homogenize the plant material for constant exposure to microwaves and heating process, which saves extraction time and increase oil yield. The machine is equipped with a water cooling system which increases the condensation efficiency and saving water. The extraction process was carried out at atmospheric pressures, 200g of crushed plant material pre-soaked in 300 mL water and then heated to 80°C for 30 minutes in the start and then subsequently increased temperatures to 90, 100 and 130°C gradients for every 30 minutes. The extraction process was continued unless maximum oil yield was obtained. The extracted oil was collected at the bottom knob of Clevenger and kept in 2mL glass tubes, dried with (Na_2SO_4), and shrouded in tinfoil, stored in the refrigerator.

2.2 Chemical analysis of extracted essential oils

The chemical analysis of essential oils was carried out by The FTIR spectra obtained using Spectrum-2 (FT-IR Spectrometer, PerkinElmer USA) interfaced with an ATR (attenuated total reflectance). Samples were fixed as a single drop of E.O. on diamond crystal and Spectra in absorbance mode measured at 600 to 4000 cm^{-1} with (Thermo Scientific Aldrich Collection of FT-IR Spectra Edition II, USA).

2.3 Isolation of causal pathogen

Isolation of disease causal pathogen was performed from infected taro leaves with typical blight symptoms (Figure 2A). leaf pieces (5mm) cut from the edge of lesions were surface-sterilized in 1.0% sodium hypochlorite for 10 minutes and washed twice with sterile distilled water; dried over sterile blotting paper, then placed on freshly prepared V8 agar medium plates and incubated at 25°C in a dark growth chamber. When the mycelium appeared from the inoculated leaf fragments, it was aseptically transferred to a new culture

media to obtain a pure culture (Fig. 2D). The isolated pathogen was identified as *Phytophthora colocasiae* keeping because of pathogenicity, colony morphology, sporangial characteristics, and microscopic observation of mycelium, as reported by (Evelyn, Charles, Grace, Estella, & Hanna, 2017; Lucas, 2020). Pathogenicity was established to fulfill Koch's postulates. For maintenance, the cultured was shifted monthly to a new media and kept at 4°C.

2.4 Antifungal evaluation of EO in vitro

2.4.1 Mycelial growth inhibition assay

The fungicidal properties of both essential oils were evaluated for isolated pathogens using the poisoned food approach following Lahlou (2004). The EO were dissolved and homogenized in sterile Dimethyl sulfoxide (DMSO) as a 9:1 (v/v) concentration before supplementation to PDA to achieve final dosages of 5, 2.5, 1.25, 0.625, 0.312, and 0.156 mg/ml. Pre-sterile 90 mm Petri plates filled with 10 ml PDA medium, with four replications for each treatment. PDA plates supplemented with only DMSO were kept as control. In contrast, fungicide (Hymexazol) was supplemented with PDA at a low concentration of 0.156 mg/ml served as a positive control for comparison. The Petri plates were inoculated with 5mm discs from the margins of a freshly produced pure culture of test fungus and incubated at 25°C in the dark growth chamber. When the Control treatment Petri plates were full of mycelium growth, the antifungal efficiency of essential oils was determined. Calculation of Mycelium growth inhibition (I) was done with formula $\{I=D_c-D_t\}$ where D_c is control and D_t is treated plates as described by Sameza (2014). The experiment consisted of four replications, and the procedure was performed again to ensure that the results. Complete inhibition of mycelium was determined by placing mycelium plugs inoculated on treated plates to fresh PDA plates for confirmation of inactive inoculum.

2.4.2 Inhibition of the sporangia production

In-vitro assessment of sporangium development on Potato Dextrose Broth medium supplemented without and with essential oils at same concentrations described above, for mycelium inhibition assay following (Tchameni et al. 2018). Five mycelial plugs (5 mm) from the center of 7 days fully grown culture of *P. colocasiae*, were homogenized in 10 mL distilled sterile water and centrifuged for 5 minutes at 4000 rpm. The sporangia in the supernatant were counted under a light microscope using a haemocytometer in 10 μ L, and inhibition was analyzed by utilizing the formula $I_s = (N_0 - N_t) / N_t \times 100$, where N_0 is the control and N_t is the oil treatment. Sporangia counted after 24 hours of incubation at 25°C, and release of zoospores was confirmed within 3 hours under fluorescent illumination. The fungicide treatments were maintained as the positive control, and only DMSO treated plates served as a negative control, whereas each treatment consisted of 4 replicates, and the trial was repeated for validation of results.

2.4.3 Inhibition of Sporangia and zoospore germination assay

Germination of Sporangia and zoospores assay was performed using the liquid dilution method suggested by Sameza (Sameza et al., 2014) with some modifications. Essential oils were homogenized in DMSO, while Hymexazol diluted in sterile distilled water was added to potato broth medium in test tubes at the previously indicated concentrations with four replications for each treatment. Medium inoculated with pathogen

suspension (500 µl) from a one-week-old culture kept 10^6 cells/mL and incubated at 25°C. Germination of zoospores after 3h and sporangia after 24h of incubation, counted under a light microscope using a hemocytometer.

2.4.4 Inhibition of symptoms and sporangia in leaf disc assay

The leaf necrosis inhibition was carried out with the leaf disc assessment method. Mature taro leaves (two months) harvested from the arboriculture garden were washed in sterile water, surface sterilized with 75% ethanol for 1 min, and rinsed thoroughly with sterile distilled water. Subsequently, the leaves were cut into a disc shape (50mm), immersed in different essential oil for 3h at a range of concentrations mentioned above, while leaf discs dipped in DMSO and Hymexazol as the negative and positive control, respectively. Individually treated leaf disc was retained in a 90mm petri dish containing moist blotting paper, 4 Petri dished for each treatment and assay repeated for confirmation of results. Thereafter leaf discs were inoculated with 5mm discs obtained from 7-day old culture and settled aseptically on the center of leaf lamina and incubated for one week in a dark chamber at 25°C. Symptoms expression was established on latency period, the diameter of necrosis, and sporangia produced by fungus on the leaf. The disease symptoms inhibition (DSI) of essential oils was assessed with the following formula: $DSI = (D_c - D_t / D_c) \times 100$, where D_t is the lesion diameter with the treatment of essential oils, and D_c is the lesion diameter in the negative control.

2.4.5 Symptoms assessment in taro corms

Fresh taro corms were harvested from the disease-free field and thoroughly washed with tap water. Uniform size corms with no visible deterioration were selected for the experiment. Thereafter surface sterilization with 1% sodium hypochlorite (NaOCl), and washed twice in sterile water and dried on blotting paper. Corms were then immersed in previously described concentrations of essential oils for 30 minutes. For pathogen inoculation, Three (5mm²) plugs were removed aseptically from the top, the central, and bottom axis of corms using a cork borer. The boreholes were injected with 5mm mycelial plugs taken from actively growing margins of pure culture of test fungus. The boreholes were sealed with the same plugs previously detached from the same corms. Four corms were used for each treatment, with and without fungicides kept as control, and the experiment was repeated. Inoculated corms were incubated at 25°C for 7 days in a dark and moist growth chamber; 7 days later, the corms were cut in vertical axis from the point of inoculation to observe symptoms of fungus invasion.

2.5 Analysis of Data

IBM (SPSS 20 Premium Campus Edition) was used for the statistical analysis. The mean±standard deviation (S.D.) represents the results. Analysis of variance (ANOVA) was used to express the connection between the variables. The least significant difference (LSD) is used to identify substantial variations in means determined at $P < 0.05$. The graphs were made using the (Graphpad Prism 9) program.

3. Results And Discussion

3.1 Chemical Composition of sage and tea tree essential oil

Both essential oils obtained by MAHD and analyzed by ATR-FTIR showed main components from sage oil as Thujone (40 %) (Fig. 3A) and tea tree oil contained Terpinen-4-ol (96%) (Fig. 3B), respectively. Prior research has found the same primary component in sage, as demonstrated by (Radulović et al., 2017), and tea tree (Elmi et al., 2019). The chemical composition of Sage and tea tree essential oils have been widely explored for its numerous pharmacological and non-toxic, and antibacterial properties (Brun, Bernabè, Filippini, & Piovan, 2019; Yazgan, 2020). Sage and tea trees have a long application history as antimicrobial and antifungal agents (Silveira et al., 2020). Therefore, these oils include a variety of oils having various characteristics, defining their importance in various sectors. Our study focuses on the antifungal properties of these essential oils, while suggestive scientific information has been extensively stated in previous experiments; similarly, revealing and proving fields of application for both oils, as well as their safe use in agriculture, should be considered appropriately.

3.2 Sage and tea tree E.O. in a mycelial inhibition assay

The outcomes of our study demonstrate that increasing the use of sage essential oil (SEO) and tea tree oil (TTO) significantly reduced the mycelium elongation of *P. colocasiae* (Fig. 4). The maximum inhibition of fungal radial colony was observed at 5.0 mg/ml for (SEO) and 1.25 mg/mL and higher for (TTO). On the other hand, fungicide fully inhibited mycelium growth at a 0.156 mg/ml dosage with the same situations. The inoculated mycelial discs from treatments with full inhibition were moved to a new culture medium to test the vitality of the mycelium, which was already dead.

The antifungal activity of sage and tea tree oils against *P. colocasiae* has been investigated for the first time. Nonetheless, there have been reports regarding the antibacterial effects of these oils. These oils have a chemically complex content of main and minor components that are important for antibacterial activity and have lately piqued the interest of researchers for their antifungal properties. The previous studies also concluded at Thujone from sage and Terpinen-4-ol from tea tree oil, which has been shown to inhibit many bacterial and fungal pathogens by affecting ATPase cell wall, biological membrane morphology, and inter-cellular metabolic pathways (Shreaz et al., 2016). However, many studies have shown that Thujone has antifungal properties towards various fungal infections by causing oxidative stress, apoptosis, epigenetic changes, and decreased toxin production (Radulović et al., 2017; Teker et al., 2021). Terpinen-4-ol has been widely reported for its antimicrobial and antifungal properties (Połec et al., 2019). Therefore, the effectiveness of the remaining minor components in sage and tea tree oil could be not be neglected. In this context, the antifungal activities of essential oils may be related to the synergistic effect of major and minor compounds (D. Wang, Zhang, Jia, Xin, & Zhai, 2019).

Furthermore, volatile chemicals produced from sage and tea plants may have a role in inhibiting mycelial growth. In general, sage and tea tree oil have been proved to be effective against *Candida albicans* (Francisconi et al., 2020), *Streptococcus agalactiae* (Zhang et al., 2018), *Sporothrix schenckii* (Brilhante et al., 2019), *Botrytis cinerea* (Li et al., 2020) *Aspergillus niger* in grapes by inducing morphous damage and metabolic changes of fungus (An et al., 2019) seed-borne fungi of cucurbits (Moumni et al., 2021)

3.3 Sage and tea tree E.O. against sporangia and zoospore germination on PDA

Compared to the controls, germination of Sporangia and zoospores was substantially decreased ($p < 0.05$) and directly correlated to oil concentration (Fig. 4). The lowest inhibitory treatment for sporangia and zoospores was 5.0 mg/mL of (SEO) and 1.25 mg/mL of (TTO), with no spore germination noticed in Hymexazol treatment. On the other hand, a significant variation seen in the morphology of sporangia and deformities in zoospores were noticed as observed under a light microscope. This is an indication of the EO-induced breaking down of the cell membrane of sporangia. Sameza (2014) investigated the antifungal effects of eucalyptus E.O. on *Pytophthora colocasiae* and described zoospores as more susceptible than sporangia (Sameza et al., 2014). Because our findings are consistent with prior research, we can explain this because zoospores lack the biological wall that sporangia do. In favorable circumstances, zoospores germinate by loss of flagellum while becoming encased in a thick wall, leading to the formation of a germ tube initiating the infection process (Matheron & Porchas, 2000). Antifungal compounds are considered to hamper with molecular mechanism of encystment and cell wall formation (Y. Bi, Jiang, Hausbeck, & Hao, 2012). The volatile components of E.O. induce vaporization, which may be responsible for this effect. Micro-molecules present in essential oils exerts synergistic action inhibiting germination due to aquaphobic characteristics, enabling permeability of cell membranes (Walker & van West, 2007). Some previous studies are evident for essential oils properties against spore inhibition of several fungi like *Aspergillus niger* and *Aspergillus flavus* (Gemed, Woldeamanuel, Asrat, & Debella, 2014) *A. oryzae*, and *A. ochraceus* (Hu et al., 2019), *Fusarium verticillioides*, and *Alternaria tenuissima* (López-Meneses et al., 2017).

Table 1
Efficacy of sage and tea tree essential oil inhibiting leaf necrosis and sporulation

	Essential oils	Latent time	Necrotic area		Sporulation on leaf	
	Concentrations mg/ml	(Hours)	Diameter (mm)	Inhibition (%)	10 ⁶ Sporangia mL ⁻¹	Inhibition(%)
Control	0.0	72	16.50±1.58a	0.00±0.00g	95.00±2.58a	0.00±0.00h
Sage	0.156	72	13.17±0.62b	20.15±3.81f	69.75±4.57b	26.57±4.8g
	0.312	=	10.60±0.62c	35.75±3.80e	43.00±3.16d	54.73±3.32e
	0.625	=	7.20±0.76d	56.36±4.60d	27.00±1.82e	71.57±1.92d
	1.25	=	4.90±0.98e	85.58±2.89b	12.75±1.70f	86.57±1.79c
	2.5	=	3.17±0.44fg	90.66±1.30b	5.75±0.95g	93.94±1.00b
	5.0	=	0.00±0.00h	100±0.00a	0.00±0.00g	100±0.00a
Tea tree	0.156	72	12.22±0.72bc	25.90±4.40f	50.75±3.5c	46.57±3.68f
	0.312	=	7.72±0.66d	53.18±4.03d	30.25±3.4e	68.15±3.58d
	0.625	=	4.00±0.41ef	75.75±2.52c	14.75±2.21f	84.47±2.33c
	1.25	=	2.15±0.34g	86.96±2.07b	0.00±0.00g	100±0.00a
	2.5	=	0.00±0.00h	100±0.00a	0.00±0.00g	100±0.00a
	5.0	=	0.00±0.00h	100±0.00a	0.00±0.00g	100±0.00a
Hymexazol	0.156	72	0.00±0.00h	100±0.00a	0.00±0.00g	100±0.00a

Values represent means of four replicates, and different letters in the same column denote significant difference as per LSD test ($P < 0.05$), Inhibition percentage compared with negative control.

3.4 Inhibition of leaf necrosis by E.O.s

Keeping in incubation for one week, noticeable blight symptoms were observed only on negative control treatments and lower applications of both essential oils (2.5 mg/mL or lower). TTO reduced necrotic signs substantially at concentrations of 1.25 and higher (Table 1). On the contrary, leaf discs treated with Hymexazol at (0.156 mg/mL) as the control treatment and E.O. applications above 5.0 mg/mL for Sage and 2.5 mg/ml for tea tree exhibited no apparent symptoms. The findings indicate a significant positive correlation between essential oil treatment and inhibition of symptoms. (Table 1). Keeping aside the mycelial invasion, zoospores germination plays a critical role during the establishment of primary infection. Our findings may be attributed to antifungal chemicals in sage and tea tree essential oils, which lysed zoospores and inhibited germination. Filomena Nazzaro (2017) described that essential oils had been regarded as having the ability to improve plant disease tolerance. The tolerance mechanism is defined as an abrupt release of the responding molecules H₂O₂, or oxidative stress, which occurs while plants are treated to any

biotic or atmospheric pressure (Nazzaro, Fratianni, Coppola, & Feo, 2017). As previously stated, the significant parts of sage and tea tree essential oil are Thujone and terpinen-4-ol, which have been shown to reduce virulence in various diseases by interference in spore germination by phosphatides dephosphorylation, and peptidases activities inhibiting germ tubes (Khan, Khan, Iqbal, Khan, & Khan, 2017). This impact may be related to how these chemicals interact with ATPase-dependent efflux mechanisms. It may also inhibit the growth of mycelia and the generation of aflatoxin, which leads to irreversible changes in hyphae formation, decreased cytoplasmic contents, and mitochondrial obliteration (Yu, Wang, Shao, Xu, & Wang, 2015). The absorption induced by these chemicals is related to the oxidation-reduction process, which regulates particular signaling systems in cells (Sun, Shang, Wang, Lu, & Liu, 2016).

3.5 Sage and tea tree E.O. against sporangia production on leaf

Results revealed that increasing applications of essential oils created a substantial reduction in Sporangia development ($p < 0.05$). (Table 1). Optimal sporangium inhibition was attained at and above (1.25 mg/mL 100% inhibition) of TTO, while SEO prevented sporangia at 5.0 mg/mL concentration. Furthermore, some morphological changes in sporangia were detected when the application E.O. was enhanced compared to the control treatment. Essential oils include a variety of chemicals that have antifungal properties against a variety of plant pathogenic fungi (D. Wang et al., 2019). These chemicals exhibit various intrusive processes for disease suppression, such as degradation of the cell-wall and lipid bilayer of biological membranes due to high permeability and release of complex biomolecules, which interact with cell membrane activities simultaneously (Shahina et al., 2018). The lipophilic nature of E.O. components enables the oil to penetrate the cell membranes, disrupt enzyme activity, and cause uncontrolled cell wall production. A high proportion of E.O.s containing volatile chemicals induces cell lysis, which inhibits the formation of sporangia (Y. Bi et al., 2012). Several studies have documented the inhibitory consequence of sage and tea tree essential oils on fungal infections; however, it is reasonable to suggest most of the volatile substances produced from the E.O. could inhibit mycelium and sporangial formation. Sporangia production of *P. colocasiae* significantly reduced using *E. globulus* E.O. at 0.625 mg/L (Sameza et al., 2014) and *C. aurantifolia* E.O. at 800 ppm concentration (Tchameni, Mbiakeu, Sameza, Jazet, & Tchoumboungang, 2018).

3.6 Sage and tea tree E.O. inhibiting symptoms in taro corms

The mycelium plugs implanted in taro corms started to degrade after few days where negative control treatment and 1.25 mg/mL essential oils or below (Figure 5). Hymexazol treatment and certain essential oil treatments did not produce disease symptoms on corms. The dark brown patches appeared near the inoculation site, which was evident that the corm surface had deteriorated. The suppression of symptoms on taro corms may be due to the high absorption of volatile oils in the fibrous mass of the corms. The increased absorption of volatile oils in the corms pith may explain the reduction of symptoms to taro corms. Taro leaf blight, *P. colocasiae*, causes serious post-harvest destruction of corms (Alexandra et al., 2020).

In comparison, oospores in corms probably stay for a long time, contributing as the massive inoculation that establishes infections eventually proceeds to epidemic scenarios (Baysal-Gurel & Cinar, 2015). Various techniques and protective and curative capabilities of plant's essential oils are being investigated for the safe and cost-effective treatment of post-harvest infections (Zheng et al., 2019). Although essential oils are often

used for sanitizing fruits and vegetables, and food items to ensure that they have a long lifetime and are pathogen-free (Sun et al., 2020)

4. Conclusion

Following the concerns of systemic fungicides that have beneficial and detrimental adverse effects on human and environmental safety, we performed this study to investigate sage and tea tree essential oil as a suitable replacement for such fungicides. Several plant essential oils with solid antifungal properties must be investigated in this context. This study may serve as a model for efficient, cost-effective, and environmentally favorable managing and controlling plant diseases with botanicals and plant essential oil-derived compounds.

Abbreviations

ATR: Attenuated total reflectance; **DDH₂O:** Double distilled water; **DMSO:** Dimethyl sulfoxide. **DRI:** Disease reduction index **EO:** Essential oil; **FTIR:** Fourier transform infrared spectroscopy; **LCD:** Liquid Crystal Display; **LSD:** least significant difference; **MAHD:** Microwave assisted hydro-distillation **MHz:** Megahertz; **PDA:** Potato dextrose agar; **PLC:** Programmable Logic Controller; derivative; **SEO:** Sage Essential Oil; **TLB:** Taro Leaf Blight; **TTO:** Tea Tree Oil

Declarations

Acknowledgment

The authors are thankful to Qingdao Fudemi biotechnology co ltd for their support.

Funding

Sichuan oil rape innovation team of national modern agricultural industry technology (SCCXTD-2021-03) supported the research.

Authors' contributions

MTK, Conceptualization, Data curation; Formal analysis and wrote the original manuscript draft; Z.H. supervised, Funding acquisition; Supervision; Validation, KGM, Visualization; Roles/Writing, F.Y., CH, W.F. and F.N. contributed in review & editing of this manuscript. All authors read and approved the final manuscript.

Competing interests

The authors have no any conflict of interest to declare

Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

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Figures

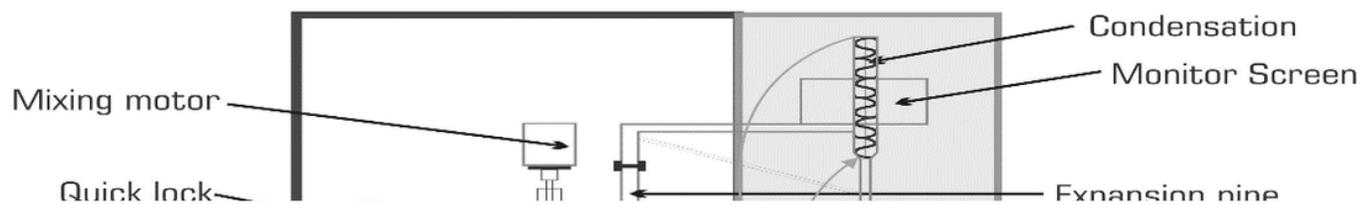


Figure 1

MAHD model design

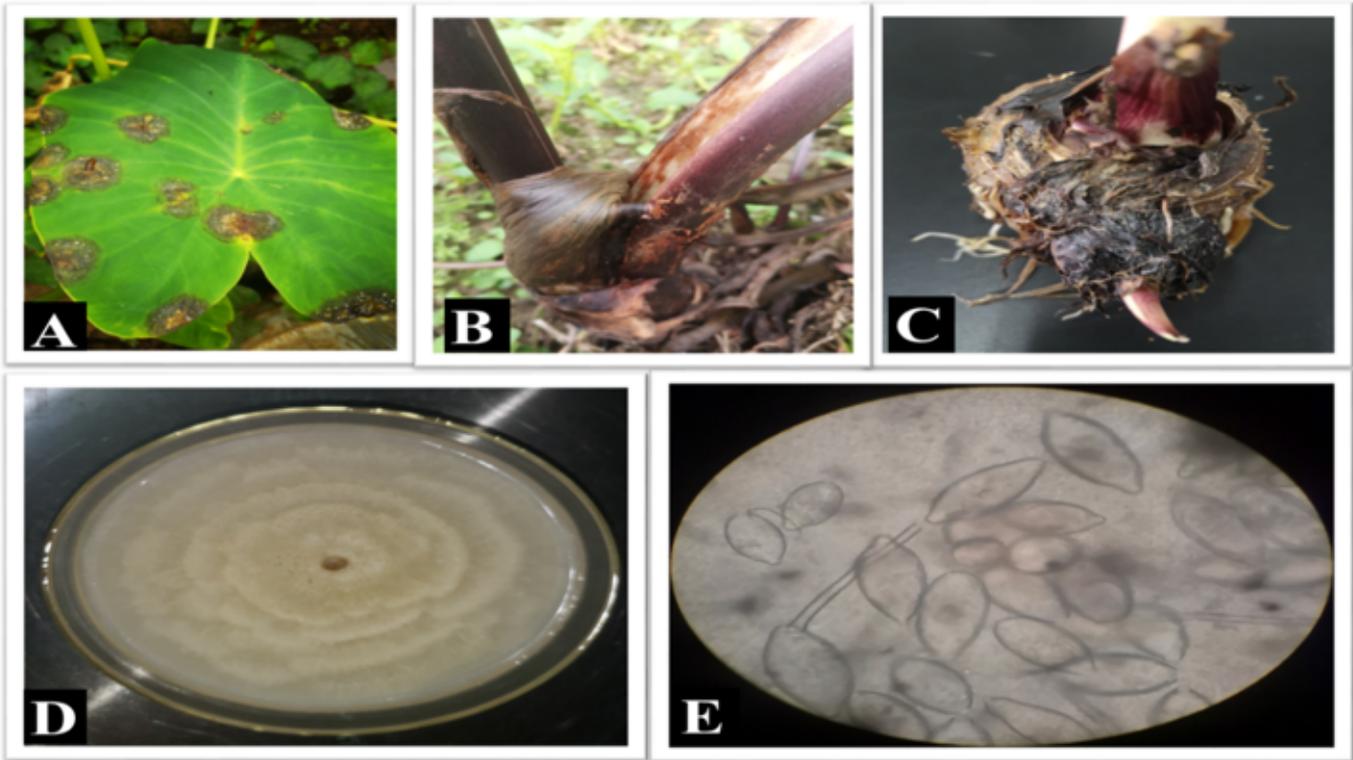


Figure 2

TLB Disease symptoms and morphology of *P. colocasiae*. **A** Characteristic Symptoms of TLB, **B**, Abrasions on leaf petioles, **C**, Infected Corms, **D**, Mycelium growth pattern **E**, Sporangia under the microscope,

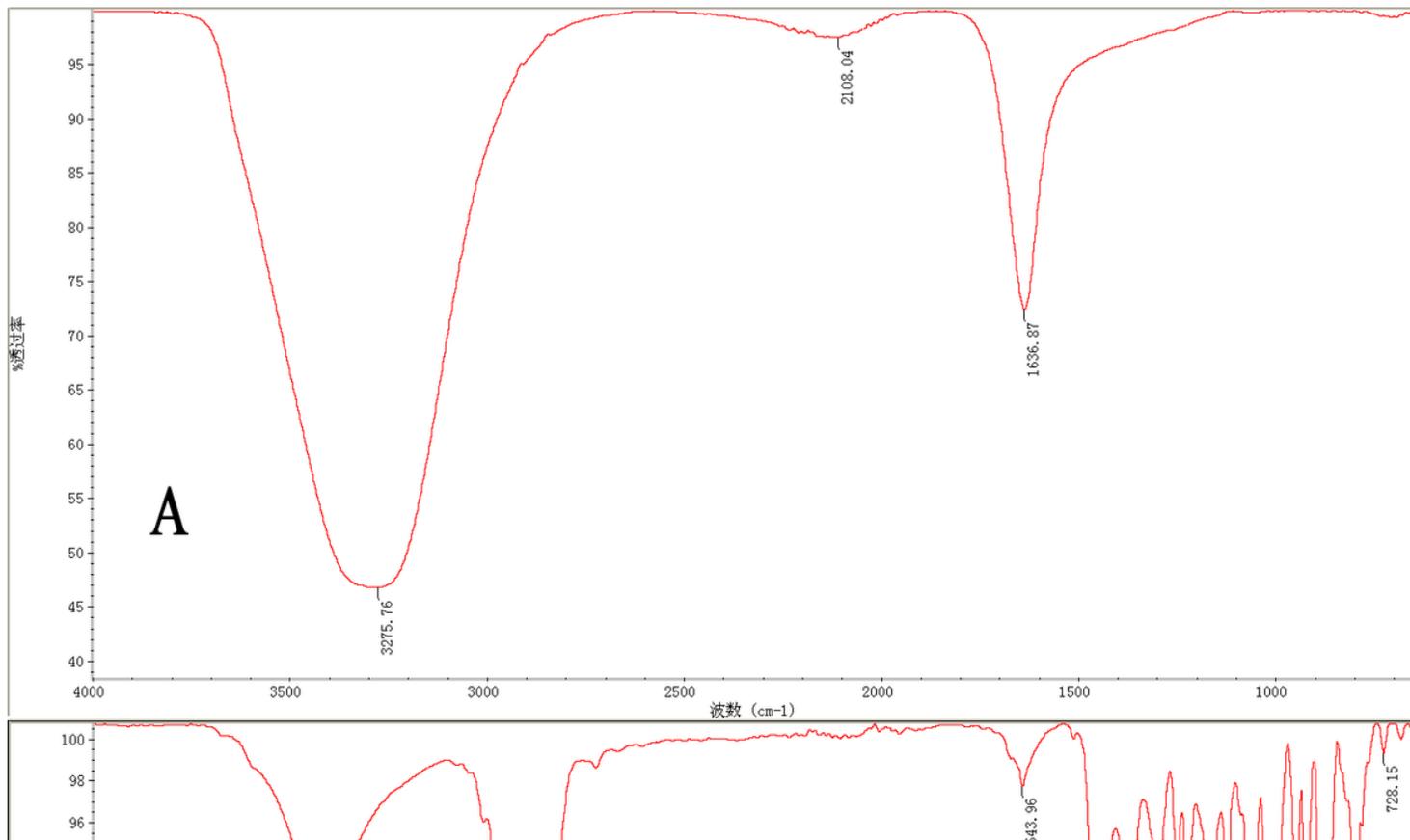


Figure 3

FTIR spectra of (a) sage and (b) tea tree essential oils

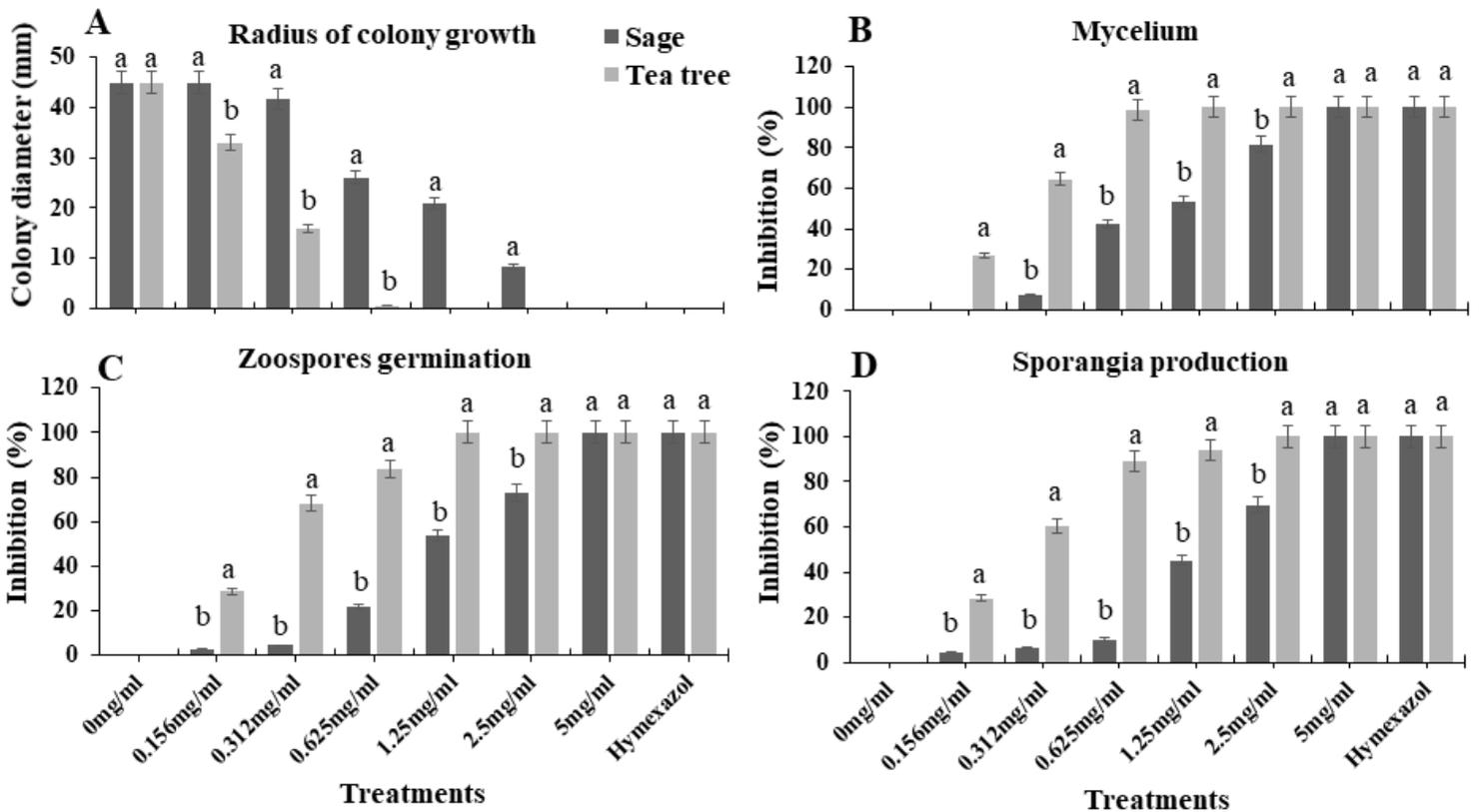


Figure 4

Efficacy of E.O.s in mycelial and spore germination inhibition

Values represent the means of four replicates, and different letters on bars denote significant differences as per the LSD test ($P < 0.05$).

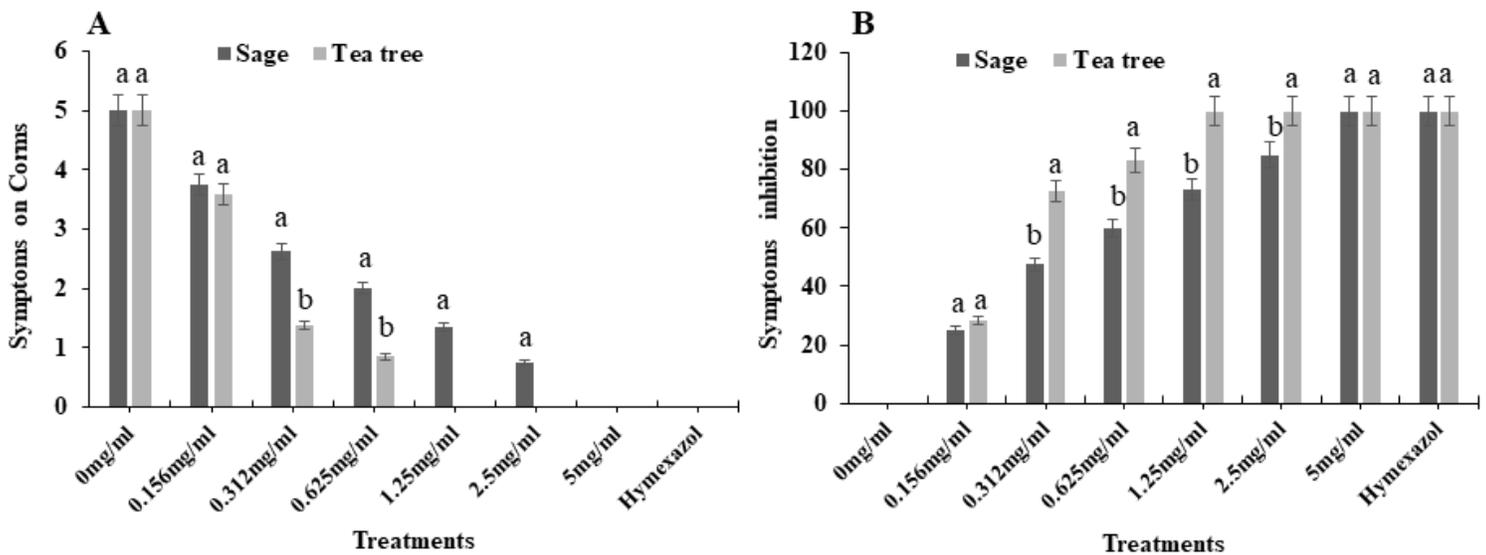


Figure 5

Efficacy of sage and tea tree E.O. inhibition of symptoms on taro corms.

Note: Dissimilar letters denote significantly different as per LSD test ($P < 0.05$)N

Symptoms measured as mycelium invasion in boreholes (mm)