Meta-analysis of essential oil effectiveness against phythopathogen in forest plant nurseries

Meta-analysis of essential oil effectiveness against phythopathogen in forest plant nurseries

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Abstract. The study aims to use a meta-analytical procedure to analyze the potential of essential oils (EO) in inhibiting the growth of pathogens *in vitro* using published data on an online database. The author accessed the online databases using search keywords, which are essential oils against phytopathogens and essential oils for fungicides to collect the article. The collected articles or sources were utilized to arrange the database. Twenty-nine studies were included to assess the inhibitory effect of EO on the growth of pathogenic mycelium *in vitro*. The collected data were analyzed statistically using a Continuous Random-Effects Model was used to ascertain a pooled effect estimate of EO treatment on mycelium inhibition. The analysis was performed using the Open Meta-analyst for Ecology and Evolution (Open MEE) software. Based on the literature review, EO in various types and concentrations can inhibit mycelium growth with standardized mean difference (SMD) = 48.734, 95% CI 45.613 to 51.855, $p < 0.001$. The results of the present study revealed the potential of EO to inhibit mycelium growth. The current meta-analysis also sets the steps for standardized experimental designs on the use of EO for bio fungicides trials in the future.

1. Introduction

The success of forest development is determined by good nursery management. Healthy seedlings will reduce the risk of failure in forest development [1]. One of the challenges in the nursery is the spread of germs due to the growth of non-solid cell walls which are vulnerable [2]. There have been many reports of disease attacks on seedlings in the nursery causing severe damage to production failure. As in the case of the Pongpolandak nursery, Cianjur, the attack of *Pestalotia sp*. is known to have caused crop failure in the pine crop in 2001 with the death of 50% of the total number of seedlings [3]. Another attack also occurred in IPB's permanent nursery in which 75% of the *Paraserianthes falcataria* seedlings aged 5 months were stricken with moderate severity [2], and many more cases.

Disease attacks can occur starting from the phase of seed, germination, and even ready-to-plant seedlings. As explained by Istikorini and Sari [2], fungi isolated from seedlings inoculated on seeds showed a percentage of disease infection of 100%. Ready-to-plant tree seedlings are generally 3 to 5 months old or more depending on the species [4]. This age is classified as susceptible to disease [2].

This condition results in damages caused by pathogens not only in the nursery but also for young plants in the field. This condition will be worsened when planting is done on agroforestry land. A pathogen can have a diverse host (cosmopolitan) which may be a pathogen for annual or seasonal crops in agroforestry systems [5].

The commonly applied management is using synthetic fungicides [6]-[8]. Excessive use causes negative impacts, including resistance [6], environmental pollution, destruction of natural enemies, the emergence of residues that endanger health, and so on [9]-[11]. Prevention efforts that can be done are utilizing biofungicides that are more effective, selective, biodegradable, and less toxic to the environment [12]. This poses the challenge of reducing the risks and negative impacts of using synthetic chemicals on human health and the environment while maintaining productivity and profitability.

Essential oils are known to be a substitute for synthetic fungicides [13]. They are a mixture of lipophilic and volatile components such as monoterpenes, sesquiterpenes, and phenylpropanoids [14],[15]. Many studies have reported the anti-fungal effect of essential oils on plant pathogens. Moleyar and Narasimham [16] stated, that several components of essential oils such as citral, cinnamic aldehyde, and citronellal can inhibit the growth of *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium digitatum*. Apart from being a single component, essential oils in various compositions also have anti-fungal properties. Essential oils of *Baccharis trimera* and *Baccharis ochracea* (*Carquejas*) at a concentration of 10 µL / mL can inhibit the growth of the pathogen *Alternaria alternata in vitro* and *in vivo* [17]. Sometimes certain types of essential oils cannot inhibit the growth of fungi, as reported by [18], at the same concentration (1%) orange (*Citrus sinensis*) and turmeric (*Curcuma longa*) essential oils are not effective in inhibiting the growth of *Aspergillus niger* compared to essential oils of clove (*Syzygium aromaticum*), lemongrass (*Cymbopogon citratus*), eucalyptus (*Eucalyptus globulus*) and mentha (*Mentha piperata*) at even lower concentrations.

The use of essential oils as a source of commercial biofungicides is very limited. In Korea, a total of 33 bio-pesticides are registered 2 (herbicides and insecticides) of which are derived from plants while the others come from microbes. There are no registered botanical fungicides in Korea [12]. The variety of essential oils used is very high. Selection of essential oils that have a high inhibiting ability for various types of pathogens in the nursery with the abundant availability and easy to develop should be further researched. To date, there has been no meta-analysis that clearly examines the use of essential oils as a biofungicide in forest plant nurseries. This current meta-analysis aims to increase the effectiveness of essential oils as an environmentally friendly source of fungicides. This study aims to use a meta-analytical procedure to analyze the potential of essential oils (EO) in inhibiting the growth of pathogens *in vitro* using published data on an online database.

2. Material and methods

2.1 Literature search

The object of meta-analysis in this study was the ability of essential oils to inhibit pathogen growth. Essential oils are expected to be a more environmentally friendly source of biofungicides. The number of relevant publications is quite high with various types of essential oils and pathogens that attack plants. This study used articles from online databases, namely Science Direct and Google Scholar, and searches for relevant articles from previously identified article sources. Keywords to look for covered essential oil against phytopathogens and essential oil for fungicides.

2.2 Data extraction and analysis

Article analysis was carried out to extract the main database by selecting articles with certain criteria. The selected articles contained information of the author, the publication year, *in vitro* experiments with pathogens that can attack forestry nurseries, and the description of the standard deviation. The data taken from the article covered the number of samples, the mean, and standard deviation of the control and treatment of each essential oil dose in the form of percent (%) inhibition. Variables or different units of units are converted into uniform units according to the information in the article. The collected data were analysed statistically using a Continuous Random-Effects Model to ascertain a pooled effect

estimate of EO treatment on mycelium inhibition. The articles that have been collected were then published in a database using Microsoft Excel software. The database that has been selected and inserted in the Microsoft Excel was saved with comma-separated values (CSV) format. The CSV format is the only format that can be used in the OpenMEE software and the output was forest plot. OpenMEE (Open Meta-analyst for Ecology and Evolution) was developed to address the need for advanced, easy-to-use software for meta-analysis, and meta-regression. This open-access software can be download at *http://www.cebm.brown.edu/openmee/help.html*.

3. Results and discussion

Essential oils (EOs) or volatile oils are secondary metabolites of a plant. Essential oils are extracted from the leaves, flower petals, stems, seeds, and even the roots of plants. Generally, EOs play important roles in direct and indirect plant defenses against herbivores and pathogens, in plant reproduction processes through the attraction of pollinators and seed disseminators, and in-plant thermo-tolerance [19]. Essential oils are increasingly in demand as a source of pesticides because of their safe status and minimal harm to health and the environment. In addition, it also reduces the development of resistant strains [17]. Many studies have revealed the ability of EOs in inhibiting the growth of pathogen both *in vitro* and *in vivo*. This current study focuses on *in vitro* inhibition.

This study selected 29 articles as presented in Table 1 . The total number of essential oils identified from the selected article was 105 types. The concentration used varies widely. Units of different variables were converted to units of ppm for statistical analysis. The diversity of pathogen types can be seen in Table 2 in which 25 types of pathogens collected from selected articles. The number of samples used was quite diverse dominated with 3 replications in 21 studies. Meanwhile, 5 studies used 4 replications, and 4 other studies used 5, 8, 10, and 21 replications of each. Each study did not mention that the pathogens used came from forestry plants as presented in Table 2 which shows the ability of the pathogens to attack forestry plants.

The overall pooled estimate for the EO effect to inhibit mycelium growth was 48.734% with a 95% confidence interval (CI) of 45.613 - 51.855, SD of 1.592, and p-values of < 0.001 as presented in Table 3, with forest plot in Figure 1. It indicates that many types of EOs can inhibit the growth of varied pathogens up to 48,734%. One article shows a fairly high degree of diversity in the effect size data with the mean value reached 100 using the essential oil treatment of *Baccharis ochracea* and *Baccharis trimera* with a concentration of 5000 and 10000 ppm. The lowest mean value with a value of 0 was found in the EO treatment of *Baccharis articulata*, *Baccharis ochracea*, and *Baccharis trimera* with concentrations of 100, 1000, and 100 ppm respectively [17]. EO concentration affects the inhibition of pathogens as showed by an experiment conducted by Thabet and Khalifa [20] and it is linear with another experiment conducted by [20] in which the inhibition was high at the lowest application rate (0.5%) and improved by increasing the tested concentration to 4% in inhibiting the pathogens *Fusarium semitectum, F. solani, F. oxysporum*, and *Rhizoctonia solani*. Besides, the inhibition was also influenced by the type of EO. The antifungal efficacy of EOs probably depends on various factors, including their chemical structure, active biological compound, concentration, and the target microorganisms [21].

The first meta-analysis on phytopathogens was carried out by Shaw and Larson 1999 [47] [25], but according to [26], the meta-analysis in plant pathology which becomes the basis is [27], since then several articles with similar methods are widely published. Several types of pathogens that attack forestry nurseries can be seen in Table 2. The genus that contributed the most species was Fusarium, including *F.circinatum, F. oxysporum, F. proliferatum, F. solani, F. subglutinans,* and *F. verticillioides*. The genus Fusarium is one of the main causes of damping-off disease [22]. For example, *Fusarium oxysporum* attack on *Pinus massoniana,* the incidence of all inoculated seedlings ranged from 80 to 100% by 30 days after inoculation [23], *F. verticillioides* and *F. oxysporum* in Pinus nigra seedlings in the Northwest Spain. Both had higher seed mortality rates than the control, namely 61.3% for *F. oxysporum* and 65.6% for *F. verticillioides* [24], and many more. These species of pathogenic fungi are potential pathogens that can inhibit the growth of seedlings and lead to the failure and death of transplanted seedlings. Damages in large numbers would be detrimental to economic [23].

Heterogeneity was evaluated through Cochrane's test (I^2 test) on the level of $\alpha = 0.10$. The value of heterogeneity was 99.926% or at a high level of heterogeneity (Table 3). If the level of heterogeneity was high, it means that the overall must be analyzed with the random-effects model or subgroup analysis [28]. Heterogeneity analysis is important in a meta-analysis because it examines the number of variants in a study group as opposed to variations in a study. A high I^2 indicates that the difference between individual study results is greater (or more variable) than expected. Excess variation may indicate that more than one outcome is being measured and therefore it may not be appropriate to combine studies for the average effect [29]. Subgroup meta-analysis can be carried out based on the active ingredient EO content. The result will show the ability of the active ingredients to inhibit the growth of pathogens. These data will be the basis for combining EO for bio fungicide. The combination can be linked to the availability and ease of cultivation. The subgroup meta-analysis can also be carried out by type of pathogen. This data can be used to analyze the most effective EO in control management to prevent serious damage according to the cause of the disease.

Table 1. Selected study as databases.

Study	Essential Oil	Phytopatogen	N
Thabet and Khalifa 2018 [20]	*Clove oil*	*Fusarium oxysporum* *Fusarium solani* *Rhizoctonia solani*	3
Youassi *et al.,* 2019 [48]	*Mondia whitei*	*Aspergillus flavus* *Penicillium sp.*	3
Tomazoni *et al.,* 2019 [17]	*Baccharis articulata* *Baccharis ochracea* *Baccharis psiadioides* *Baccharis trimera*	*Alternaria alternata*	10
Lopez- meneses *et al.,* 2017 [49]	*Cinnamon* *Lemongrass*	*Fusarium verticillioides*	3
Romagnol *et al.,* 2010 [50]	*Cuminum cyminum*	*Botrytis cinerea* *Fusarium oxysporum* *Pythium ultimum* *Alternaria spp.*	3
Safari *et al.,* 2011 [51]	*Satureja richingeri*	*Rhizopus stolonifer*	5
Sethi *et al.,* 2015 [52]	*Alpinia allughas*	*Sclerotium rolfsii* *Rhizoctonia solani*	3
Pandey *et al.,* 2013 [53]	*Adhatoda vasica Nee* *Aegle marmelos* *Anisomeles indica* *Annona squamosa* *Asphodelus tenuifolius* *Azadirachta indica A. Juss* *Callistemon lanceolatus* *Chenopodium ambrosioides* *Chrysanthemum indicum* *Citrus aurantium* *Cleome gynandra*	*Aspergillus flavus* *Aspergillus niger* *Alternaria alternata* *Curvularia lunata* *Fusarium oxysporum* *Penicillium sp.*	3

	Cotula anthemoides *Curcuma aromatica* *Cyperus triceps* *Erigeron bonariensis* *E. canadensis* *Gynura crepidioides* *Hygrophila difformis* *H.pinnatifida* *Lawsonia inermis* *Leucas cephalotes* *Melia azedarach* *Murraya koenigii* *M.paniculata* *Piper longum* *P.methysticum* *P.sylvaticum* *Pogostemon heyneanus* *P.plectranthoides* *Psidium guajava* *Putranjiva roxburghii* *Saraca indica* *Syzygium cumini* *Tagetes erecta* *Xanthium strumarium*		
Fratelnale *et al.,* 2014 [54]	*Angelica archangelica*	*Fusarium oxysporum* *Fusarium solani* *Fusarium verticillioides* *Botrytis cinerea* *Alternaria solani*	3
Wenqiang *et al.,* 2006 [55]	*Artemisia argyi*	*Botrytis cinerea* *Alternaria alternata*	3
Kumar *et al.,* 2013 [56]	*Morina longifolia*	*Fusarium solani* *Alternaria alternata* *Aspergillus flavus* *Aspergillus fumigatus*	3
Moutassem *et al.,* 2019 [57]	*Thymus pallescens* *Cymbopogon citratus* *Schinus molle* *Laurus nobilis* *Artemisia herba* *Pinus halepensis*	*Fusarium oxysporum*	4
Gakuubi *et al.,* 2017 [58]	*Eucalyptus camaldulensis*	*Fusarium solani* *Fusarium oxysporum* *Fusarium verticillioides* *Fusarium proliferatum* *Fusarium subglutinans*	3
Sethi *et al.,* 2013 [59]	*Ocimum basilicum* *Ocimum kilimandscharicum* *Ocimum gratissimum* *Ocimum sanctum*	*Rhizoctonia solani*	3

Tchoumbougnang *et al.,* 2009 [60]	*Satureja robusta* *Satureja punctata*	*Aspergillus niger*	21
Kumar *et al.,* 2014 [61]	*Nepeta leucophylla* *Nepeta ciliaris* *Nepeta clarkei* *Calamintha umbrosa*	*Fusarium oxysporum* *Rhizoctonia solani* *Alternaria solani*	3
Mena rodriguez *et al.,* 2018 [62]	*Lippia alba*	*Macrophomina phaseolina*	3
Rahman *et al.,* 2011 [63]	*Cymbopogon citratus* *Piper chaba*	*Colletotrichum gloeosporioides* *Fusarium oxysporum* *Fusarium solani* *Rhizoctonia solani*	3
Curini *et al.,* 2003 [64]	*Erigeron canadensis* *Myrtus communis*	*Rhizoctonia solani* *Fusarium solani*	8
Espana *et al.,* 2017 [65]	*Eucalyptus camaldulensis* *Eucalyptus globulus* *Eucalyptus tereticornis*	*Colletotrichum gloeosporioides*	3
Lee *et al.,* 2008 [66]	*Eucalyptus citriodora* *Melaleuca quinquenervia* *Leptospermum petersonii*	*Phytophthora cactorum*	4
Kim *et al.,* 2008 [67]	*Pimenta racemosa (bay)* *Juniperus oxycedrus* *Cymbopogon nardus* *Pelargonium graveolens* *Cuminum cyminum* *Myristica fragrans* *Cymbopogon martini* *Mentha pulegium* *Mentha spicata* *Thymus vulgaris*	*Phytophthora cactorum* *Cryponectria parasitica*	4
Znini *et al.,* 2013 [68]	*Pulicaria mauritanica*	*Alternaria sp.* *Rhizopus stolonifer*	3
Feng and Zheng 2006 [69]	*Cassia oil* *Thyme oil*	*Alternaria alternata*	3
Rahman *et al.,* 2010 [70]	*Erigeron ramosus*	*Fusarium oxysporum* *Colletotrichum capsici* *Fusarium solani* *Rhizoctonia solani*	3
Lee *et al.,* 2009 [71]	*Artemisia arborescens* *Chamomile. roman* *Coriander. herb* *Cypriol* *Myrrh* *Pastinak* *Patchouli* *Peru Balm. distilled* *Salvia stenophylla* *Sandalwood AGD*	*Phytophthora cactorum* *Cryphonectria parasitica* *Fusarium circinatum*	4

	Santolina *Spikenard. chin* *Oriental sweetgum* *Valerina. ind* *Verbena* *Manuka (wild)* *Texas-cedar* *Carrotseeds* *Cypress. blue*		
Al reza *et al.,* 2010 [72]	*Cestrum nocturnum*	F. oxysporum F. solani C. capsici R. solani B. cinerea	3
Mishra and Dubey 1994 [73]	*Cymbopogon citratus*	aspergillus flavus	3
Dubey *et al.,* 2007 [74]	*Eupatorium cannabinum*	Botryodiplodia theobromae Colletotrichum gloeosporioides	4

Table 2. Pathogens and forestry hosts species in the meta-analysis.

No	Genus	Species	Host	Reference
1	Alternaria	*Alternaria alternata*	*Ailanthus excelsa*	[30]
			Eucalyptus globulus	[31]
2		*Alternaria solani*		
3		*Alternaria spp.*	*Akasia*	
4	Aspergillus	*Aspergillus flavus*	*Pinus roxburghii*	[32]
5		*Aspergillus fumigatus*		
6		*Aspergillus niger*	*Pinus roxburghii*	
7	Botryodiplodia/	*Botryodiplodia*	*Pinus Caribaea*	[33]
	Lasiodiplodia	*theobromae*	*Hevea brasiliensis*	[34]
8	Botrytis	*Botrytis cinerea*	*Pinus sylvestris L*	[35]
9	Colletotrichum	*Colletotrichum capsici*	*Dalbergia latifolia*	[36]
10		*Colletotrichum gloeosporioides*		
11	Cryponectria	*Cryphonectria parasitica*	*Castanea dentata*	[37]
12	Curvularia	*Curvularia lunata*	*Dalbergia sissoo*	[38]
13	Fusarium	*Fusarium circinatum*	*Pinus patula*	[39]
14		*Fusarium oxysporum*	*Pinus nigra*	[24]
15		*Fusarium proliferatum*	*Pinus lambertiana*	[40]
16		*Fusarium solani*	*Melia dubia*	[41]
17		*Fusarium subglutinans*	*Pinus merkusii*	[42]
18		*Fusarium verticillioides*	*Pinus nigra*	[24]
19	Macrophomina	*Macrophomina phaseolina*	*Pinus radiata*	[43]
20	Penicillium	*Penicillium sp.*	*Leucaena leucocephala*	[44]
21	Phytophthora	*Phytophthora cactorum*	*Pinus*	
22	Pythium	*Pythium ultimum*	*Pinus*	

23	Rhizoctonia	*Rhizoctonia solani*	*Paraserianthes falcataria*	[2]
24	Rhizopus	*Rhizopus stolonifer*	Tectona Grandis *Gmelina Arborea*	[45]
25	Sclerotium/ Athelia	*Sclerotium rolfsii*	*Pterocarpus santalinus* *Swietenia macrophylla*	[46]

Table 3. Summary result.

Estimate	Lower bound	Upper bound	Std. error	p-Value	I^2
48.734	45.613	51.855	1.592	< 0.001	99.926

4. Conclusion

Essential oils are expected to be biofungicides in the future. Many studies have reported the ability of essential oils to inhibit the growth of pathogens. Meta-analysis can be an effective tool to determine the effectiveness of control using essential oils. The high heterogeneity among these studies holds back any definite conclusions. The research using sub-group analysis and meta-regression is needed, to determine which essential oils have the good inhibitory capacity and which pathogens are the wariest of when attacking forestry nurseries.

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