Antifungal properties of essential oils for improvement of indoor air quality: a review

Harriet Whiley*, Sharyn Gaskin, Tiffany Schroder and Kirstin Ross

Abstract: Concerns regarding indoor air quality, particularly the presence of fungi and moulds, are increasing. The potential for essential oils to reduce, control or remove fungi, is gaining interest as they are seen as a “natural” alternative to synthetic chemical fungicides. This review examines published research on essential oils as a method of fungal control in indoor environments. It was difficult to compare the relative performances of essential oils due to differences in research methods and reporting languages. In addition, there are limited studies that scale up laboratory results and assess the efficacy of essential oils within building environments. However, generally, there appears to be some evidence to support the essential oils clove oil, tea tree oil, oregano, thyme and lemon as potential antifungal agents. Essential oils from heartwood, marjoram, cinnamon, lemon basil, caraway, bay tree, fir, peppermint, pine, cedar leaf and manuka were identified in at least one study as having antifungal potential. Future studies should focus on comparing the effectiveness of these essential oils against a large number of fungal isolates from indoor environments. Studies will then need to focus on translating these results into realistic application methods, in actual buildings, and assess the potential for long-term antifungal persistence.

Keywords: fungicidal; indoor air; natural; plant-derived compound; plant extract.

Introduction

Indoor air quality is a public health issue of increasing concern (1–3). One of the leading indoor air quality complaints is the presence of fungi and moulds, which have been associated with increased risk of adverse health effects (4, 5). The most common adverse health effects associated with fungi in indoor environments, as recently reviewed by Nevalainen et al. (6), are various respiratory conditions. Other potential health effects include allergic responses, infection or toxigenic effects, for which the pathophysiology is less evident (2). Of these, infection and toxicity have serious consequences but are rare in occurrence, whereas allergic responses are commonly observed (7). These allergic responses include rhinitis, eye irritation, cough and aggravation of asthma, which is of emerging significance given that the incidence of asthma in children from developed countries is increasing (8–10).

Burr et al. (11) conducted a randomised control trial to explore the effect of eradicating visible mould from the homes of asthma patients. It was found that the symptoms of asthma and rhinitis improved and medication usage decreased in the patients who had indoor mould removed, fungicide applied and a fan installed in the loft of their homes.

Fungi and moulds have also been demonstrated to contribute to sick building syndrome and other building related illnesses (7, 12). Sick building syndrome is recognised as a group of symptoms (e.g. eyes, nose and throat irritation; dry skin, headache and lethargy) that are related to spending time in a particular building. This is more commonly identified in offices; however, there have been reports of sick building syndrome in schools, hospitals, care homes and domestic houses (13). Research has demonstrated that one of the key risk factors of sick building syndrome is dampness, which promotes mould growth (14).

To reduce the potential risk of exposure, indoor areas with visible fungal growth must be immediately remediated (15). Early intervention for fungal contamination is essential; otherwise, professional remediation will be required (3). Remediation typically involves the removal...
of building material with visible mould contamination, in conjunction with treatment of surfaces with an antifungal product. Remediation should also include steps to prevent moisture build-up, which enables future fungal growth (16). An antifungal agent (otherwise known as a fungicide) is a compound used to kill or inhibit the growth of fungi and/or fungal spores (sporicide) (17). Antifungal agents recommended for indoor environments should be non-toxic to humans, odourless and hypoallergenic (18). It is ideal that the antifungal agent also provides long-term protection from fungal regrowth, especially in humid or moist environments that would promote fungal growth; however, in reality, this long-term persistent protection is difficult to achieve especially for non-toxic fungicides (19, 20).

Globally, there is increasing concern regarding synthetic chemical usage and residue and the potential human health effects of exposure (21). Consequently, there is a push from consumers for ‘natural’ alternatives to chemical antifungal products for use in residential and commercial indoor environments (2, 15, 22). As such, research on essential oils and their potential antimicrobial capabilities has received increasing attention (23). Essential oils are complex mixtures of volatile compounds biosynthesised by plants, the main groups of which include terpenes and terpenoids and aromatic and aliphatic constituents, and are characterised by low molecular weight (24). Essential oils have been widely used in medicine and the food industry for their antimicrobial properties; however, there are limited studies investigating their use for the control of indoor air quality. The increased interest in natural substances is driving the research community to find new applications of these substances. The aim of this review was to examine studies that have investigated the antifungal potential of essential oils specifically as a method for improving indoor air quality for building occupants.

Methods

A search was conducted through the Scopus (25), ProQuest (1), Science Direct (6) and Web of Science (26) databases. The search terms included (mould OR mold OR fungi OR fungal OR fungus) AND (“indoor air” OR indoor OR building OR buildings) AND (“essential oil” OR “essential oils” OR “plant-derived compound” OR “plant extract”) AND (antifungal OR fungicidal OR fungicide OR sporicidal OR sporicide OR anti-microbial OR biocide OR biocidal), and the search was limited to the title, abstract and keywords. Figure 1 presents the systematic approach to article inclusion or exclusion. Articles were screened by reading titles and abstracts and initially excluded if they did not describe original research or did not examine the fungicidal activity of essential oils. Articles were then read in full and excluded if they described a fungal control not relating to indoor air or buildings (e.g. articles describing the control of clinical isolates, animal, crop or food spoilage, etc. were excluded). A total of 19 studies that described the antifungal potential of essential oils or their extracts for the purpose of influencing indoor air quality were included and are summarised in Table 1.

Antifungal potential of essential oils for the control of indoor air environments

The biggest challenge when evaluating the antifungal potential of essential oils or their extracts (Table 1) is the lack of a standard method for both designing experiments and describing antifungal efficacy (43). The most commonly adopted screening method identified in the studies shown in Table 1 was the disk diffusion assay. This is where the essential oil or treatment is added to filter paper discs and placed in the centre of agar plates containing fungal lawn. The zone of clearing in fungal growth is measured as an indicator of fungal inhibition. Other studies also used modified versions of this method, including adding the essential oil through syringe injection or placing in a well in the centre of the agar plate (26, 36, 37). Rogawansamy et al. (15) used the disk diffusion assay in addition to a method adapted from Soylu et al. (44) to investigate the antifungal efficiency of the treatments in the vapour phase. Briefly, agar plates with fungal lawns are created and filter paper containing the treatment is placed on the inner surface of the agar plate lid, ensuring no direct contact with the fungal lawn. Plates are sealed with parafilm and antifungal efficiency is determined by measuring the zone of clearing. Another method frequently used was the serial dilution method, where serial dilutions of essential oils were prepared and inoculated with fungal cultures to determine the minimum concentration that inhibited fungal growth (25, 32, 40).

Even when the same method was used, it is difficult to compare results as a consequence of different reporting language used to describe antifungal efficacy. Using the disk diffusion assay, researchers reported the results as either diameter of zone of inhibition (15, 37) or as percentages of growth inhibition compared with the control plates (25, 29, 33). Using the serial dilution method, Šegvić Klarić et al. (40) reported antifungal efficacy using the term minimum inhibitory concentration (MIC), which is the lowest concentration that allowed no more than 20% fungal growth.
Whiley et al.: Indoor air and antifungal essential oils

(determined by a reduction in the number of colonies in 10 μL of the dilution inoculated onto Sabouraud Glucose Agar incubated at 25°C for 7 days), and minimum fungicidal concentration (MFC), which was the lowest concentration of essential oil that completely inhibited the growth of the fungi. However, this differs from the definitions used by Stupar et al. (32), who also used a variation of the serial dilution method but reported the MIC as the lowest concentration without visible growth (assessed using a binocular microscope) and the MFC as the lowest concentration with no growth after inoculation of the original inoculum. This definition of MIC was supported by Verma et al. (45), who reported MIC as the lowest concentration that resulted in no growth after the incubation period.

The other challenge when evaluating the antifungal efficacy of essential oils is that most of the studies identified in Table 1 are laboratory based and there is a lack of in situ experiments within building environments. This makes it difficult to translate the experimental findings into ‘real-world’ recommendations. Only one study, by Su et al. (23), investigated the antifungal efficacy of essential oils by evaporating essential oil in indoor rooms and measuring the changes in air quality. Another four studies assessed the antifungal efficacy of essential oils against fungal growth on different types of wood surfaces used in building construction; however, all other studies identified were conducted on agar plates or broth cultures (36–39). There is clearly need for further research designed to investigate the antifungal efficacy in indoor environments (in situ) in order to validate the translation of laboratory-based outcomes. There is also a need to investigate the potential long-term persistence of the treatment and any optimum reapplication requirements in order to characterise antifungal capabilities.

Clove oil

Of the essential oils identified in Table 1, clove oil has been researched the most extensively, and there are a
Table 1: Studies investigating the antifungal activity of essential oils for the control of fungi in indoor environments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fungal species</th>
<th>Efficacy</th>
<th>Strengths</th>
<th>Weaknesses</th>
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<tr>
<td>Extracts from heartwood (Pinus rigida), eucalyptus leaves (Eucalyptus camaldulensis) and creper ginger rhizomes (Costus speciosus)</td>
<td>Alternaria alternata, Fusarium subglutinans, Chaetomium globosum, Aspergillus niger and Trichoderma viride</td>
<td>Wood specimens treated at the level of 2% concentration of heartwood extract observed good inhibition to the mould growth</td>
<td>– Moderate transferability of results to real-world application (wood blocks were immersed in the treatment prior to placing on agar plates inoculated with fungal species)</td>
<td>– Unknown relevance of fungi to environmental isolates (stock cultures used)</td>
<td>(27)</td>
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<td>Extracts from marjoram (Origanum majorana), thyme (Thymus vulgaris) and ginkgo leaves (Ginkgo biloba)</td>
<td>C. globosum (ATCC 6205), A. niger (ATCC 9642), Aureobasidium pullulans (ATCC 15233), Gliocladium virens (ATCC 9645) and Penicillium pinophilum (ATCC 11797)</td>
<td>Marjoram extracts demonstrated excellent antifungal performance in the laboratory experiments and when applied to the antifungal mortars</td>
<td>– Efficacy compared to commercial chemical antifungal agents</td>
<td>– Unknown relevance of fungi to environmental isolates (ATCC strains used)</td>
<td>(28)</td>
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<tr>
<td>Extracts from myrrh (Commiphora myrrha)</td>
<td>Acremonium strictum, Aspergillus flavus, Aspergillus sydowi, C. globosum, Cladosporium cladosporioides, Cladosporium sphaeroporum, Cladosporium verruculoscladosporioides, Cochliobulus spicifer, Drechslera biseptata, Embellisia chlamydospora, Eurotium astloadami, Fusarium semitectum, Myceliophthora lutea, Penicillium chrysogenum, Penicillium felutum, Penicillium reticulatum, Phoma tropica, Torula caligans, Trichoderma pseudokoningii and Ulocladium consortiale</td>
<td>The antifungal efficacy of myrrh was highly dependent on the sensitivity of the fungal species. The highest growth inhibition (74.6%) was against A. strictum and the lowest growth inhibition (12.7%) was against U. consortiale</td>
<td>– Relevant fungal species used (isolated from indoor air environment)</td>
<td>– Limited transferability of results to real-world application (growth inhibition percentage calculate using disk diffusion assay)</td>
<td>(25)</td>
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<td>Cavicide® and Virkon®, 70% ethanol, vinegar (4.0%–4.2% acetic acid) and tea tree oil (<em>Melaleuca alternifolia</em>)</td>
<td><em>Aspergillus fumigatus</em> and <em>P. chrysogenum</em></td>
<td>Tea tree oil demonstrated the greatest inhibitory effect on the growth of both fungi, followed by Cavicide® and Virkon®. Vinegar only inhibited <em>P. chrysogenum</em> and 70% ethanol had no inhibitory effect</td>
<td>– Relevant fungal species used (isolated from indoor air environment)</td>
<td>– Limited transferability of results to real-world application (growth inhibition zones were measured using disk diffusion assay on agar and antifungal activity in vapour phase was assessed using by placing a treated filter paper disk on the inside lid of an agar plate containing a fungal lawn)</td>
<td>(15)</td>
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<td>Clove oil (<em>Syzygium aromaticum</em>)</td>
<td>Three white-rot fungi (<em>Trametes hirsuta, Schizophyllum commune,</em> and <em>Pycnoporus sanguineus</em>) (common causes of wood rot)</td>
<td>50 μg/g clove essential oil had 100% mortality to <em>Reticulitermes chinensis</em> after testing for 5 days</td>
<td>– Efficacy compared to commercially available fungicides</td>
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<td>Cinnamon oil (<em>Cinnamomum verum</em>) and clove oil (<em>S. aromaticum</em>)</td>
<td><em>Aspergillus</em> sp. (WU1003), <em>Trichothecium</em> sp. (WU1004), <em>Trametes</em> sp. (WU1005) and <em>Gloeophyllum</em> sp. (WU1006)</td>
<td>3% concentration of cinnamon and clove oil gave complete inhibition against <em>Aspergillus</em> sp. and <em>Trichothecium</em> sp. on rubberwood particleboard for 9 weeks. Particleboards treated with clove or cinnamon oil were found to have reduced mass loss from <em>Trametes</em> sp. and <em>Gloeophyllum</em> sp. compared to the controls. The percentage loss of mass decreased with increasing levels of clove and cinnamon oils</td>
<td>– Moderate transferability of results to real-world application (particleboards were treated and then dipped into the mould spore inoculum and then incubated in a humid environment)</td>
<td>– Unknown relevance of fungi to environmental isolates (stock cultures used)</td>
<td>(29)</td>
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<td>Cinnamon oil (C. verum) and clove oil (S. aromaticum)</td>
<td><em>Aspergillus</em> sp. WU1003 and <em>Trichothecium</em> sp. WU1004</td>
<td>Dip treatment in cinnamon oil and clove oil at a concentration of 0.63% was capable of providing complete protection for at least 8 and 5 weeks, respectively. Contents of cinnamaldehyde and eugenol in the particleboards, the main antifungal agents in cinnamon oil and clove oil, respectively, largely declined within the first 4 weeks of incubation, which could explain the time limit of the fungicidal activity</td>
<td>– Moderate transferability of results to real-world application (particleboards were treated and then dipped into the mould spore inoculum and then incubated in a humid environment)</td>
<td>– Unknown relevance of fungi to environmental isolates (stock cultures used)</td>
<td>(31)</td>
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<tr>
<td>Oregano (<em>Origanum vulgare</em>) essential oil</td>
<td><em>A. fumigatus</em>, <em>Aspergillus nidulans</em>, <em>Aspergillus versicolor</em> and one <em>Penicillium</em> species</td>
<td>Oregano demonstrated strong antifungal activity against all fungal isolates tested; however, it was not as effective as biocide and benzalkonium chloride</td>
<td>Relevant fungal species used (isolated from indoor air environment)</td>
<td>Limited transferability of results to real-world application [antifungal activity was assessed using the suspension-neutralisation method on malt extract agar and the agar microdilution technique (results reported as MIC and MFC)]</td>
<td>(32)</td>
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<td>Essential oils from caraway (<em>Carum carvi</em> L.), bitter orange (<em>Citrus aurantium</em> L.), bergamot orange (<em>Citrus bergamia</em> Risso &amp; Poit), coriander (<em>Coriandrum sativum</em> L.), common juniper (<em>Juniperus communis</em> L.), English lavender (<em>Lavandula angustifolia</em> Mill.), com mint (<em>Mentha arvensis</em> L.), pennyroyal (<em>Mentha pulegium</em> L.), basil (<em>Ocimum basilicum</em> L.), lemon basil (<em>Ocimum citriodorum</em> Vis), marjoram (<em>O. majorana</em> L.), oregano (<em>O. vulgare</em> L.), bay tree (<em>Pimenta racemosa</em> (Mill.) W. Moore), rosmary (<em>Rosmarinus officinalis</em> L.), common sage (<em>Salvia officinalis</em> L.), sage (<em>Salvia sterea</em> L.), tansy (<em>Tanacetum vulgare</em> L.), thyme (<em>Thymus satureoides</em> Coss. &amp; Balansa., <em>T. vulgaris</em> L.) and ginger (<em>Zingiber cassumunar</em> Roxb.)</td>
<td>The group of most effective essential oils including coriander, lemon basil, oregano, caraway, bay tree and thyme achieved high inhibition levels up to 100% across the entire spectrum of target pathogenic fungi</td>
<td>High antifungal activity</td>
<td>Low antifungal activity: Bitter orange, bergamot orange, common juniper, basil, common sage, tansy and ginger all failed to reach 50% antifungal inhibitive effect in a single target fungi</td>
<td>– Relevant fungal species used (isolated from indoor air and surfaces of water damaged buildings)</td>
<td>– Limited transferability of results to real-world application (agar microdilution method on potato dextrose agar. Antifungal activity was reported as percentage of growth inhibition compared to control.)</td>
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<td>Siberian fir (<em>Abies sibirica</em> L.), common caraway (<em>Carum carvi</em> L.), peppermint (<em>Mentha piperita</em> L.), willow-leaved gum tree (<em>Eucalyptus globulus</em> Labill.), lemon thyme (<em>Thymus pulegioides</em> L.), clove tree (<em>S. aromaticum</em> (Linn.) Merrill &amp; Perry) and bergamot orange (<em>C. bergamia</em> Risso &amp; Poit)</td>
<td>The highest fungicidal activity was demonstrated by clove oil. All cultures were affected and the fungicidal zones ranged from 20 to 50.5 mm. This fungicidal activity was comparable to the most effective disinfectant (BioSheen 20.9–57.5 mm). Next, fir oil was also effective against all fungi tested, but its fungicidal impact was less than that of clove oil</td>
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<td>– Efficacy compared to commercial disinfectants</td>
<td>– Incubation time was describes as 3–5 days. The effect of time was not assessed</td>
<td>(34)</td>
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| Crude extract of glycoalkaloids from nightshade (Solanaceae) plants | *Ulocladium chartarum*  
In addition, the fungus *Penicillium digitatum* was found spreading on the wall in one case | The extract of glycoalkaloids from nightshade plants demonstrated only partial growth inhibition of *Fusarium* and *Rhizoctonia* genera | – Relevant fungal species used (isolated from building surfaces)           | – Limited transferability of results to real-world application (percentage of growth inhibition was calculated using the agar micro-dilution method)  
– Incubation time was described as 4–5 days. The effect of time was not assessed  
– Efficacy was not compared to commercial fungicide |
Thujopsene (found in the essential oil of a variety of conifers) | Highest antifungal activity shown by clove, lemon, bitter orange and peppermint. The concentration of 5 ppm was as effectively as 5 ppm Ketoconazole (positive control) and the lowest was shown by castor oil, cedar and olive | – Relevant fungal species used (isolated from indoor air and surfaces)  
– Efficacy compared to commercially available fungicide as positive control | – Limited transferability of results to real-world application (MIC were determined using potato dextrose agar plate needle-inoculated in the centre with the antifungal agent)  
– Multiple time points were not assessed |
| Thujopsene (found in the essential oil of a variety of conifers) | *A. niger*, *Aspergillus ochraceus*, *A. sydowii*, *Aspergillus ustusa*,  
*Botrytis cinerea*, *Eurotium herbarionum*, *Gonytrichum macrocladum*, *Penicillium decumbens*, *Penicillium expansum*, *Penicillium hirsutum*, *Penicillium polonicum*, *Penicillium sp.*, *Periconia britannica*,  
*Rhizopus stolonifer*,  
*S. chartarum* and *Ulocladium botrytis* | Thujopsene demonstrated fungicidal activity against only 5 of the 16 fungal strains tested | – Relevant fungal species used (isolated from indoor air environment)  
– Fungicidal ability tested against a large number of fungal isolates  
– Multiple time points assessed | – Limited transferability of results to real-world application (disk diffusion and agar well diffusion assay on malt extract agar and fungicidal activity reported as growth inhibition zones)  
– Efficacy not compared to commercially available fungicide | (35) |

**Table 1 (continued)**
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<tr>
<td>Cedar leaf oil (<em>Thuja plicata</em>)</td>
<td><em>Candida albicans</em> and <em>A. niger</em></td>
<td><em>C. albicans</em> was readily killed by cedar leaf oil. <em>A. niger</em> was inhibited but complete eradication was not achieved.</td>
<td>– Moderate transferability of results to real-world application (dried films of fungi were exposed directly to cedar leaf oil) – Multiple time points assessed</td>
<td>– Unknown relevance of fungi to environmental isolates (ATCC strains used) – Efficacy not compared to commercially available fungicide</td>
<td>(38)</td>
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<tr>
<td>Essential oil extracts from manuka (<em>Leptospermum scoparium</em>) including eugenol, thymol, cinnamaldehyde, carvacrol, manuka oil, manuka oil less triketones fraction and triketones</td>
<td><em>Penicillium corylophilum</em>, <em>A. alternata</em> and <em>Cladosporium herbarum</em></td>
<td>Eugenol, cinnamaldehyde, thymol and carvacrol completely inhibited the growth of the three test fungi at a concentration of 1% w/v. All the extracts, including eugenol, cinnamaldehyde and thymol virtually completely inhibited the growth of <em>P. corylophilum</em> on unfinished gypsum board at 3% w/v and significantly reduced growth on the finished gypsum boards.</td>
<td>– Moderate transferability of results to real-world application (initial experiment – growth inhibition was examined on malt extract agar containing essential oil compared to growth of the control. Follow-up experiment – the efficacy of eugenol, thymol and cinnamaldehyde was evaluated against <em>P. corylophilum</em> on gypsum board. Finished (sealed and painted) and unfinished gypsum was sprayed with the essential oil treatment and then inoculated with 10^7 spores/mL) – Multiple time points assessed</td>
<td>– Source of fungal isolates not reported – Efficacy not compared to commercially available fungicide</td>
<td>(39)</td>
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<td>Thyme essential oil and thymol</td>
<td><em>Aspergillus</em> spp., <em>A. versicolor</em>, <em>A. niger</em>, <em>A. sulphureus</em>, <em>A. flavus</em>, <em>P. chrysogenum</em>, <em>P. brevicompactum</em>, <em>P. griseofulvum</em>, <em>Penicillium</em> spp., <em>Alternaria</em> spp., <em>Absidia</em> spp., <em>Mucor</em> spp., <em>C. gloeosporioides</em>, <em>S. chartarum</em></td>
<td>Both thymol and thyme essential oil showed strong fungicidal activity. Vapor phase of the thyme essential oil at concentration of 82 μg L⁻¹ exhibited fungi-static and/or fungicidal activity during 60 days of exposure in glass chambers. They were also demonstrated to be sporicidal against all tested mould species.</td>
<td>– Relevant fungal species used (isolated from building surfaces) – Moderate transferability of results to real-world application [initial experiment – MIC and MFC were determined by using the serial broth dilution method. Follow-up experiment – the activity of the vapour phase of essential oil of thyme was tested by a modified micro-atmosphere method]</td>
<td>– Efficacy was not compared to commercially available fungicide – Multiple time points were not assessed</td>
<td>(40)</td>
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| Essential oils from lemon \((C. limon)\) including \(C. paradisi, C. sinensis, Citrus aurantifolia\) and \(Citrus reticulate\) | \(A. niger\) | \(*\) | \(C. aurantifolia and C. reticulata exhibited significant antifungal potency against building fungi\) | – Source of fungal isolates not reported  
– Multiple time points not assessed  
– Efficacy was not compared to commercially available fungicides  
– Limited transferability of results to real-world application (MICs were calculated at different concentrations of essential oils on potato dextrose agar) | (41) |
| Volatile organic compounds \((VOCs)\) produced from evaporating essential oils indoors lavender \((L. angustifolis)\), eucalyptus \((E. globulus)\) and tea tree \((M. alternifolia)\) | Natural fungi contained within air samples | A slight decrease in fungal concentrations was observed in the first 30–60 min but levels quickly increased to pre-treatment concentrations | – Comparatively transferable to real-world application \([300 \mu L of each essential oil was diluted with 50 mL water for use in incense evaporator with burning candle in two rooms \((21.6 \text{m}^3 \text{ and } 28.2 \text{m}^3)\). Changes in total airborne fungal concentration were examined by air sample collection using Burkard sampler and enumerated on malt extract agar plates an hour prior to treatment]\)  
– Relevant source of fungi used  
– Multiple time points assessed  
– Efficacy not compared to commercially available fungicide | (23) |
| Essential oil from pine tree \((Pinus sylvestris L.)\) | \(A. niger, Penicillium funiculosum, P. chrysogenum, T. viride, Ulocladium oudemansii, Paecilomyces variotii, Phoma glomerata, S. chartarum and A. versicolor\) | Pine oil displayed fungicidal activity against all fungi tested, although the effectiveness depended on the fungal species and the concentration of pine oil | – Limited transferability to real-world application \([MICs were determined using oil diffusion on Czapek agar (for fungi) and malt extract agar (for yeast and yeast-like fungi)]\)  
– Multiple time points not assessed  
– Efficacy not compared to commercially available fungicide | (42) |

DMSO, Dimethyl sulfoxide; ATCC, American type culture collection.
number of studies (laboratory and in situ) that have demonstrated that clove oil has strong antifungal capabilities. The most robust study demonstrated that clove essential oil had fungicidal activity comparable to commercial disinfectants and had the highest antifungal efficacy compared with Siberian fir, common caraway, peppermint, willow-leaved gum tree, lemon thyme and bergamot orange against fungi isolated from indoor air and surfaces (A. versicolor, A. niger, A. fumigatus, C. sphaerospermum, C. cladosporioides, P. chrysogenum, P. aurantiogriseum, P. simplicissimum, U. chartarum and P. digitatum) (34). Another study that was designed to have moderate transferability of results, by Yingprasert et al. (31), demonstrated that particle board that had been dipped in 0.63% clove oil completely inhibited Aspergillus and Trichothecium for up to 5 weeks. By increasing the concentration to 3%, it was found that Aspergillus and Trichothecium were inhibited for up to 9 weeks and the percentage of mass lost as a consequence of Trametes and Gloeophyllum was reduced by 5% (31).

These findings were supported by other studies that used agar plates spiked with clove oil to demonstrate that it had higher antifungal efficacy against A. niger and G. candidum compared with black pepper, castor-oil plant, cedar, eucalyptus and olive (36). Also, a study using the agar spiked method found that clove oil was 100% effective at controlling R. chinensis, a common white rot fungi found in wood surfaces (29).

Tea tree oil

Two studies from Table 1 identify tea tree oil as a potential antifungal agent. The most translatable study was Su et al. (23), which evaluated tea tree oil by evaporating it in a room and measuring the changes in fungal concentration using an air sampler. This study is one of the few investigations into applying essential oils as a fungicide in a real-world building environment. It was found that the concentrations of fungi in the air initially decreased as a result of the VOCs from tea tree oil, but after 30–60 min, the concentrations returned to normal background indoor levels. However, one of the limitations of this study was that it did not compare the efficacy of tea tree oil to that of commercially available fungicides. Another study used the disk diffusion assay method to test tea tree oil against A. fumigatus and P. chrysogenum isolated from indoor air samples and found that it had greater fungicidal activity compared with some commercially available antifungal agents (15).

Oregano

Oregano was identified as an effective antifungal agent in a study by Zabka et al. (33) using the agar microdilution method. It was demonstrated to have high inhibition levels against all fungi tested (A. alternata, S. chartarum, C. cladosporioides and A. niger) and was more effective compared to other essential oils, including English lavender, pennyroyal, corn mint, sage, bitter orange, bergamot orange, common juniper, basil, common sage, tansy and ginger. However, this study used stock culture fungi, which makes it difficult to translate these results compared to studies using environmentally isolated fungi, and did not compare efficacy to that of commercially available fungicides. These findings were supported by another study that also used the microdilution methods to demonstrate that oregano displayed antifungal properties against A. fumigatus, A. nidulans, A. versicolor and a Penicillium species isolated from the frescoes within a monastery in Serbia. However, it was not as effective compared to the biocide benzalkonium chloride, a quaternary ammonium compound (32).

Thyme

Thyme was also identified as an effective antifungal agent in a study by Zabka et al. (33), with greater fungal inhibition potential compared to essential oils from English lavender, pennyroyal, corn mint, sage, bitter orange, bergamot orange, common juniper, basil, common sage, tansy and ginger. However, this study used stock culture fungi, which makes it difficult to translate these results compared to studies using environmentally isolated fungi, and did not compare efficacy to that of commercially available fungicides. It was also demonstrated to have fungicidal and sporicidal activity in both the liquid and vapour phases against fungal isolates collected from the walls of damp buildings in Slovakia (40). However, in other more robust studies, it was shown to be not as effective compared to clove oil, fir oil or marjoram (28, 34).

Citrus

Verma et al. (41) demonstrated that the volatile essential oils from lime (C. aurantifolia) and mandarin (C. reticulate) exhibited significant antifungal potency against building fungi A. niger. This was followed up by another study using a modified disk diffusion assay method (the essential oil was needle-inoculated in the centre of the agar
plate), which showed that 5 ppm concentration of lemon essential oil was as effective as 5 ppm ketoconazole (a synthetic antifungal drug) against *A. niger* and *G. candidum* isolated from the surfaces and indoor air of buildings. The study also demonstrated essential oil from lemon to have greater antifungal potential compared to castor oil, cedar and olive (36).

**Other essential oils demonstrating fungicidal potential**

Other essential oils that demonstrated potential antifungal activity in at least one study (Table 1) include heartwood, marjoram, cinnamon, lemon basil, caraway, bay tree, fir, peppermint, pine, cedar leaf and essential oil extracts from manuka (eugenol, cinnamaldehyde, thymol and carvacrol).

**Essential oils demonstrating only moderate or low fungicidal activity**

The essential oils identified in Table 1 which demonstrate only moderate or limited antifungal activity include eucalyptus leaves, crêpe ginger, ginkgo leaves, myrrh, English lavender, pennyroyal, corn mint, sage, bitter orange, bergamot orange, common juniper, common basil, nightshade, castor-oil-plant, olive, willow-leaved gumtree and thujaopsene (a compound found in the essential oil of a variety of conifers).

**Mechanism of antifungal activity**

Understanding the mechanism(s) of action of different antimicrobial agents is important to characterise efficacy as one agent may not inhibit all microorganisms. It is important to acknowledge the principal differences between bacteria and fungi. The structures of fungi and bacteria differ in significant ways, for example most fungi are diploid in nature and have longer generation time compared with bacteria (46). This means that antibacterial and antifungal agents target structures and functions most relevant to the organisms to be inhibited. For example, many antibacterial agents inhibit steps important for the formation of peptidoglycan (47), the essential component of the bacterial cell wall. In contrast, most antifungal compounds target either the formation or the function of ergosterol (48, 49) an important component of the fungal cell membrane. This membrane interaction weakens the structure, increasing permeability, which is responsible for the leakage of solutes across the membrane and causes cell lysis. For example, Shao et al. (50) described this mechanism of action when applying tea tree oil on *B. cinerea* (an important fungus in viticulture and food spoilage). Tea tree oil was found to inhibit the growth of the fungus and germination of spores was suppressed. The cell wall structure was reported to have lost its ultrastructure and showed thickening and rupturing. The authors concluded that the cell wall integrity was destroyed, increasing the membrane permeability.

Overall, there is currently limited knowledge regarding the antimicrobial mechanisms of essential oils, particularly with regards to antifungal activity (36, 49). A few authors have mentioned the antimicrobial activity of essential oils; however, the mechanism of action has not been studied in great detail (49, 51). Chemical analysis of essential oils show that the major active components are phenols, terpenes, aldehydes and ketones (52), and it is generally believed that essential oils principally act against cell cytoplasmic membranes of microorganisms. Hydrophobicity is an important characteristic of essential oils and their components (51), which may enable them to accumulate in cell membranes, disturbing the structures and causing an increase of permeability.

One study by Pinto et al. (49) demonstrated that clove oil and eugenol (the main component of many essential oils) was found to be fungicidal as a result of extensive lesion of the fungal cell membrane. In addition, clove oil and eugenol reduced the quantity of ergosterol, a specific fungal cell membrane component. This resulted in inhibition of germ tube formation of *C. albicans* (49). Similarly, it has been suggested that the antifungal action of tea tree oil is as a result of its capability to change or damage the function of fungal membranes (50, 53).

A great deal remains to be learned about the mechanisms of action of essential oils against fungal species. Although some progress has been made with clinical investigations, a greater understanding of these mechanisms is clearly lacking for other environmental organisms. Studies of the mechanisms of action relevant to fungal species in indoor air would allow more efficient and effective use of these agents.

**Potential health effects – is ‘natural’ safer?**

The increasing interest in ‘natural’ products for controlling microorganisms in indoor environments is due in part to the perception of benefit (i.e. inhibition of fungal growth) without the need for using potentially ‘harmful synthetic
chemicals’. However, this assumption that ‘natural’ products are not harmful to human health is flawed.

Currently, there are limited studies investigating the potential adverse health consequences of repeated exposure to essential oils. The oils themselves are complex mixtures, which may contain naturally occurring contact sensitisers. In fact, some evidence suggests that they are potential skin allergens or sensitising agents (23, 54, 55). An ideal antifungal agent would not generate toxic fumes during application and is non-irritating if accidentally exposed to skin. Skin irritation and skin sensitisation are different responses; skin irritation occurs on the first exposure to the agent; the inflammatory reaction is typically rapid and the severity will depend on the concentration of the irritant present, compared with skin sensitisation, which is a complex allergic immunological response, with the reaction typically occurring after repeated exposure to the chemical and is usually irreversible (i.e. once sensitised, always react). Schaller and Korting (55) described a case report of allergic contact dermatitis due to repeated exposure to essential oil use in aromatherapy (applied topically or released as aerosols). There have also been several studies that have demonstrated exposure to essential oils exacerbated respiratory problems including asthma, decreased pulmonary function and increased chest tightness (56, 57).

Thus, the perception that ‘natural’ is safer may not necessarily be appropriate when considering essential oils for fungicidal treatment, and care must be taken with their repeated application in the indoor environment. Essential oils should be considered in the same way that use of chemical fungicides would be, based on risk assessment.

Conclusion and recommendations

Fungal contamination of indoor buildings and indoor air quality is a health issue of increasing concern. There is a need for greater guidance regarding appropriate antifungal agents to treat fungal contamination of buildings, as well as a drive from consumers and other groups to consider the potential of essential oils as a ‘natural’ alternative to commercially available fungicides. This review identifies the studies assessing the fungicidal potential of essential oils against fungi relevant to indoor air quality. The biggest challenge with comparing the fungicidal efficacy of essential oils from different studies is the lack of a standard method and reporting language. Additionally, the efficacy of essential oils is also dependant on the fungal species being challenged which makes it difficult to compare studies using different fungal isolates.

However, despite these challenges, clove oil was identified as the best-performing essential oil within the more robust studies. Additionally, there appears to be some evidence to support the essential oils tea tree oil, oregano, thyme and lemon as potential antifungal agents with relevance to indoor air quality. Heartwood, marjoram, cinnamon, lemon basil, caraway, bay tree, fir, peppermint, pine, cedar leaf and manuka were also identified in at least one study as having antifungal potential; however, there is a need for more robust studies to examine these further.

Future studies should focus on comparing the efficacy of these essential oils against a large number of fungal isolates from indoor environments. Studies will then need to focus on translating these results with in situ studies investigating the effectiveness in actual buildings and assessing the potential for long-term antifungal persistence. The studies identified in this review, which were either moderately or comparatively translational (23, 28, 31, 38–40), can inform the design of these studies. However, they should additionally compare the efficacy of essential oils to commercially available fungicides and examine the effect of time on fungicidal activity. Furthermore, when considering the application of these essential oils in building environments, the effect of different concentrations, mechanisms of application and the potential human side effects must also be examined.

Author Statement

Research funding: Authors state no funding involved.
Conflict of interest: Authors have no conflicts of interest to declare. Informed consent: Informed consent is not applicable. Ethical approval: The conducted research is not related to either human or animal use.

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