Antifungal activity of essential oils against selected building fungi

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Fungi can cause damage to the structures, decoration of buildings and are also responsible for their indoor air quality. In this study, the antifungal activity of essential oils of selected plant species, viz. *Piper nigrum* Linn., *Ricinus communis* Linn., *Cedrus deodara* (Roxb.) Loud., *Syzygium aromaticum* (Linn.) Merrill & Perry, *Eucalyptus globulus* Labill., *Citrus aurantium* Linn., *C. limon* (Linn.) Burm. f., *Olea europaea* Linn. and *Mentha piperita* Linn. were assayed for fungi toxicity against two genus, viz. *Aspergillus niger* and *Geotrichum candidum*. The highest and broadest activity was shown by the essential oils of *S. aromaticum, C. limon, C. aurantium* and *M. piperita*, while the oils of *R. communis, C. deodara* and *O. europaea* demonstrated the lowest level of antifungal activity among the oil tested as compared to standard drug, Ketoconazole. The 5 ppm concentration of essential oils of *S. aromaticum, C. limon* and *M. piperita* completely inhibited the mycelial growth of *A. niger* and *G. candidum* to the same extent as 5 ppm of Ketoconazole. However, the 5 ppm concentration of essential oil of *C. aurantium* completely inhibited the mycelial growth of *G. candidum* at 10 ppm concentration to the same extent as 5 ppm concentration of Ketoconazole in positive control. Knowledge gained through this research may lead to find out the safer, biodegradable, renewable, cheaper, target specific alternative of restricted fungicides for fungi management in buildings.

Keywords: Antifungal, Building fungi, Essential oils, Minimum inhibition concentration.

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Introduction

Fungi are a natural part of our environment and play an important role in decomposition of organic matter. They can grow on almost any building material if enough moisture is available and cause damage to the structures, decorations of buildings and are also responsible for the indoor air quality.1

The *Penicillium* sp., yeast, *Cladosporium* sp. and *Aspergillus versicolor* are commonest fungi in both mould-damaged and non-damaged buildings. Several fungal genera detected in damaged and control buildings include *Aspergillus* spp. (*A. penicillioides, A. sydowii, A. fumigatus, A. nidulans, A. ustus, A. ochraceus, A. glaucus*), *Arthrographis* sp., *Aureobasidium* sp., *Engyodontium* sp., *Eurotium herbariorum, Geomyces pannorus, Phoma sp., Stachybotrys chartarium* and *Wallenia sebi*.2 Fungi have a widespread distribution in buildings in temperate regions of Asia, Europe, North America, and Oceania.3 In air and surface samples from hospitals, offices and residential buildings of Roorkee, India, *Alternaria, Aspergillus, Penicillium, Rhizopus* and *Trichoderma* species were found present in wall of all types of buildings. However, *Aspergillus, Geotrichum, Mucor, Penicillium* and *Rhizopus* species were found in indoor environment.4,5

Studies over the past hundred years have demonstrated the antimicrobial properties of several common spice oils. Maruzzela and Balter7 found that 100 essential oils out of 119 spice oils tested possessed an antagonistic effect to at least 1 of 12 pathogenic fungi and 50 of these samples showed a wide spectrum of activity against all fungi tested. However, little is known about the antifungal activity of essential oils against building fungi which are known to cause damage to structure and decoration of interior and exterior surface of buildings, their indoor environment and occupants. In the present study nine essential oils were distilled from various plant materials belonging to different families and also investigated for their antifungal activity against *A. niger* and *G. candidum* commonly cause deterioration on buildings and their indoor environment.

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Materials and Methods

Essential oils extraction

The fresh leaves of *Eucalyptus globulus* Labill. (Myrataceae), *Mentha piperita* Linn. (Lamiaceae), *Olea europaea* Linn. (Oleaceae), *Cedrus deodara* (Roxb.) Loud. (Pinaceae), dried seeds of *Piper nigrum* Linn. (Piperaceae), fresh seeds of *Ricinus communis* Linn. (Euphorbiaceae), dried buds of *Syzygium aromaticum* (Linn.) Merrill & Perry (Myrtaceae) and peels of *Citrus limon* (Linn.) Burm. f. and *C. aurantium* Linn. (Rutaceae) were collected/purchased from the flowering plants/superstore for essential oil extraction. The plant materials were identified and voucher specimens were submitted in the herbarium of Building Pests and Mycology Laboratory, Environmental Science and Technology Division, CSIR-Central Building Research Institute, Roorkee, India. Plant materials were thoroughly washed twice with distilled water and subjected to Clevenger’s hydro-distillation apparatus to isolate essential oils. The isolated essential oil was dehydrated with anhydrous sodium sulphate (100 mg/ml) and kept on stand for half an hour. There after, upper layer containing dehydrated essential oil was collected with the help of micropipette and stored in clean glass vial at 4°C (Ref. 8).

Chemicals and culture media

The standard antibiotic Ketoconazole (Sigma-aldrich, New Delhi, India) powder was prepared in dimethyl sulfoxide (Sigma-aldrich, New Delhi, India) and stored at -20°C. Potato dextrose agar (Himedia) was used for broth micro dilution assay.

Microorganisms

The *A. niger* and *G. candidum* were isolated from the surface and indoor air of building, respectively4,5 and have been maintained on potato dextrose agar (Himedia) in Building Pests and Mycology Laboratory, Environmental Science and Technology Division, CSIR-Central Building Research Institute, Roorkee, India.

Minimal inhibitory concentration (MIC) determination

All the essential oils were individually evaluated for their fungal toxic activity against *A. niger* and *G. candidum* by adopting food poisoning technique into a petridish9,10. For each fungal isolate, a conidial spore suspension of 10⁶ spores/ml was prepared and petridishes were needle-inoculated in the centre. The oils have been tested at 5, 10, 20, 30, 40 and 50 ppm concentration prepared by dissolving in dimethyl sulfoxide (DMSO). Ketoconazole (standard antibiotic) dissolved in DMSO was used as a positive control. The potato dextrose agar (Himedia) nutrient medium was poisoned with appropriate concentration of test essential oil. The radial growth was measured in millimeters after incubated at 28 ± 0.5°C and 85% relative humidity for 96 h in triplicate. The minimal inhibitory concentration (MIC) was determined as the lowest concentration of oil which results no growth of the inoculum on all petridish11. MIC was defined as the lowest oil concentration showing no visible growth after incubation time.

Results and Discussion

The antifungal activity of various essential oils on the growth of *A. niger* on potato dextrose agar is presented in Figure 1. The essential oils of...
S. aromaticum, C. limon, C. aurantium and M. piperita exhibited highest antifungal activity (MIC) at 5 ppm of concentration after incubation of 96 h as compared to standard (Ketoconazole). The essential oils of P. nigrum and E. globulus exhibited moderate antifungal activity (MIC) at 30 ppm of concentration. However, the essential oils of R. communis, C. deodara and O. europaea exhibited least antifungal activity as compared to standard antibiotic.

The antifungal activity of various essential oils on the growth of G. candidum on potato dextrose agar is presented in Figure 2. The essential oils of S. aromaticum, C. limon, and M. piperita exhibited highest antifungal activity (MIC) at 5 ppm of concentration after incubation of 96 h as compared to standard. The essential oil of C. aurantium exhibited MIC at 10 ppm of concentration. The essential oils of P. nigrum and E. globulus exhibited moderate antifungal activity (MIC) at 20 ppm of concentration. However, the essential oils of R. communis, C. deodara and O. europaea exhibited least antifungal activity as compared to standard antibiotic.

Fig. 2.—Antifungal activity of various essential oils against Geotrichum candidum after 96 hours of incubation. The results are presented as the mean ± SE of three independent experiments in triplicate.
Conclusions
The results of present study showed that essential oils of *S. aromaticum*, *C. limon* and *M. piperita* exhibited highest antifungal activity on the growth of *A. niger* and *G. candidum*, which are commonly found in buildings and their indoor environment. The present study will be helpful in the realistic approach for the development of eco-friendly fungicide/antifungal paint and thus in the effective management of fungi development in buildings and indoor environment. Further, long term studies are under way to isolate and characterize the major active principles of the oils and test the components on different fungi on building materials.

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References