

## Anti-fungal potency of essential oils with coating for surface treatment of heritage Solani Aqueduct, Roorkee, India

Rajesh K. Verma\* and Neeraj Jain

Building Pests and Mycology Laboratory, Building Material and Environmental Sustainability Group, CSIR-Central Building Research Institute, Roorkee 247667, Uttarakhand, India

Received 29 April 2022; revised received 12 April 2023; accepted 16 May 2023

The Solani Aqueduct is 175 years old heritage structure in Roorkee, India. The exterior surface of structure is deteriorated due to fungal infestation. Fungi can spoil valuable heritage sites aesthetically, mechanically and chemically. Five fungi namely: *Curvularia pallescens*, *Fusarium equiseti*, *Talaromyces purpureogenus*, *Aspergillus niger*, and *Alternaria alternata* were isolated and identified from exterior surface of the Solani Aqueduct. Essential oils (Eucalyptus, Peppermint, and Clove) were evaluated by using agar well diffusion method against three (out of five) dominating fungi *A. niger*, *T. purpureogenus*, and *A. alternata* to determine the zone of inhibition. The clove oil (10000 ppm) exhibited anti-fungal activity against different fungi in the following order i.e., *Alternaria*>*Talaromyces*>*Aspergillus*. Further, clove oil (10000 ppm) with three individual water-repellent coatings (konex 2318, konex 2319, and zycosil-zycoprime) was applied on a white cement panel to determine their anti-fungal performance against above three fungi. The clove oil (10000 ppm) exhibited effective results against all three fungi up to 28 days. The GC-MS spectra of clove oil exhibited eugenol content of 86.95%. The maximum depth of penetration of coating was determined using various dyes and was recorded as maximum for konex 2319 (3.0 mm) followed by konex 2318 (2.7 mm) and zycosil-zycoprime (1.3 mm). On the basis of visual observation and water hydrophobicity test performed on field trial at surface of Solani Aqueduct, Roorkee, the order of effective protecting coating has been in order off clove oil (10000 ppm) with konex 2319 >konex 2318 >zycosil - zycoprime.

**Keywords:** Cement panel, Essential oil, Fungal deterioration, Heritage building, Solani aqueduct

**IPC code; Int. cl. (2021.01)-** A61K 36/00

### Introduction

The Solani Aqueduct is an old upper Ganga canal, built in 1846 at Roorkee, Uttarakhand, India. It is considered a heritage site because of its brick masonry (lime mortar) structure that is built over the Solani River. It is 980 feet long aqueduct consisting of 15 spans of 50 feet each, which is separated by 10 feet wide piers (Fig. 1). Due to heavy moisture conditions its exterior surface is deteriorated by fungal infestation. The impact of fungal activity on deterioration of cultural heritage is a global problem and their preservation over time is a challenging task<sup>1</sup>. Fungal problems in both modern and historic buildings are attributed to environmental conditions such as water, humidity, temperature and a lack of ventilation favouring the decay of materials<sup>2</sup>. Fungi play a considerable role in the deterioration of cultural heritage. Water activity ( $a_w$ ) determines the

ability of microorganism to grow. Due to their enormous enzymatic activity and their ability to grow at low  $a_w$ , fungi are able to inhabit and decay paintings, textiles, paper, parchment, leather, oil, casein, glue, and other materials used for historical art objects<sup>3</sup>.

Essential oils are usually complex mixtures of natural compounds<sup>4</sup>. These are used for their antimicrobial activity in food products<sup>5-7</sup>. They can represent one of the most promising natural products for fungi control<sup>8</sup>. The essential oils obtained from different plants or herbs exhibited intense antifungal properties<sup>6,8-11</sup>. Eucalyptus and peppermint oils were found effective against plant pathogenic fungi<sup>12-13</sup>. Menthol was found to be an individual aroma responsible for the antifungal activity of peppermint essential oil<sup>14</sup>. The peppermint essential oil exhibited strong antifungal activities against examined fungi at concentrations ranging from 0.12 to 8.0  $\mu\text{L}/\text{mL}$ <sup>15</sup>. Anti-fungal activities of essential oil and methanolic extracts of peppermint were demonstrated<sup>16-23</sup>. The

\*Correspondent author  
E mail: rkverma\_cbri@cbri.res.in

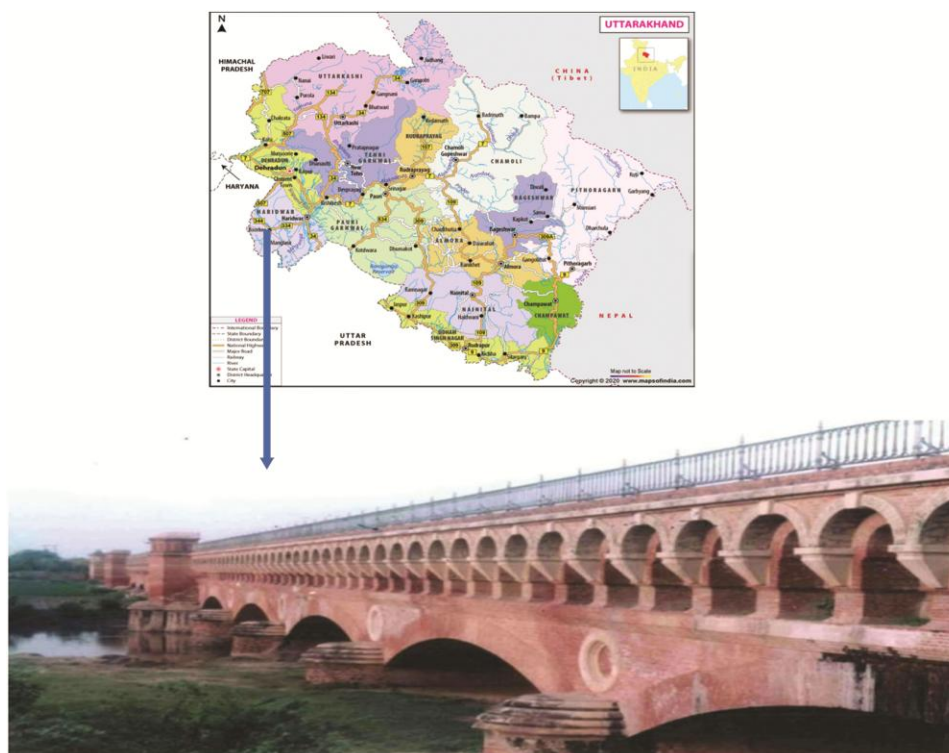


Fig. 1 — Location map and exterior view of heritage Solani aqueduct, Roorkee, Uttarakhand.

essential oils from three *Eucalyptus* species showed significant antifungal activity against *Sclerotium rolfsii* and *Fusarium solani*<sup>24</sup>. The essential oil of *Eucalyptus* possesses good anti-fungal potential against a few fungal strains<sup>25-26</sup>. Antifungal activity and minimum inhibitory concentration (MIC) of tested essential oils with different concentrations (0.125, 0.25, 0.5, 0.75, 1  $\mu\text{L/mL}$ ) were determined<sup>1</sup>. The potency of various essential oils against fungal growth on cultural heritage was reviewed<sup>27</sup>. The main phytochemical composition of eucalyptus, peppermint and clove oils are summarized<sup>28</sup>.

Water-repellent coatings are commonly used to protect culturally significant works, such as outdoor sculptures and architectural elements. These coatings are applied to heritage structures to protect them from surface damage, corrosion, and staining. They either block the pores in the surface to reduce the absorption of water and salts or form an impermeable layer which prevents such materials from passing<sup>29</sup>. The three individual protecting coated with peppermint and eucalyptus oils were applied on a cement panel to evaluate their anti-fungal activity against *Mucor racemosus* for up to 21 days and mixed culture of *Rhizopus stolonifer*, *Penicillium chrysogenum*, and *Mucor racemosus* up to 28 days<sup>30</sup>. Transparency and

stability of the visual aspect of coatings are very important factors to fulfil heritage conservation criteria<sup>31</sup>.

Commercially available water-repellent coatings are based on silicon, silane, silicate, silicate, acrylic, epoxy, urethane, etc. However, these were not assayed for their anti-fungal activity against fungi occurring in heritage structures. Many chemical compounds (oxidizing agents, organic substances, and metals) are used to control the deteriorating fungal growth. Increased awareness of the harmful effects caused by use of fungicides has led to an interest in cleaner residue-free technologies<sup>1</sup>. The alternative natural conservation processes should be safe, easy to use, and economical without any side effects on heritage sites. The aim of the present investigation is to identify the prominent fungal species responsible for the bio-deterioration of lime mortar surface and to determine the performance of protecting coatings with essential oils for surface treatment of heritage Solani Aqueduct, Roorkee, India.

## Materials and Methods

### Location and heritage site for sample collection

The Solani aqueduct is situated in Roorkee, Haridwar District of Uttarakhand, India. The aqueduct

over the Solani River is considered amongst the first of its kind in India and is known remarkably for its construction; truly in accordance with the repute that Roorkee holds for engineering. The water bridge is 172 feet wide and 24 feet tall, and once two lions sat at one end to mark the beginning of the canal's irrigation area (Fig. 1).

#### Isolation and identification of deteriorated fungal species

The fungal samples were collected during August-October, 2021, by swab and fungi tape method from the different surface areas of the heritage Solani Aqueduct, Roorkee. The sites or walls which have been continuously moist or damaged for a long period of time were chosen for the sampling (Fig. 2). Collected fungal samples were kept in sterile polythene and sealed immediately to avoid contamination. These were taken to the laboratory in an ice box for subsequent fungal isolation/identification and stored at 4°C in the refrigerator. The collected fungal samples were cultured on potato dextrose agar (PDA) medium. The petri plates were incubated in a BOD incubator at 25°C for 72 h. After 72 h of incubation, different coloured fungal growth was observed on PDA petri plates (90 mm). The different coloured fungal colonies exhibited mixed fungal culture on Petri plates. The mixed fungal culture was further sub-cultured to prepare pure individual culture. Streaking was done by picking culture from each coloured fungal growth on a fresh PDA petri plate. The pure cultured fungi were utilized for their morphological identification. After 7 days

pure cultures of fungus were isolated. The slides were prepared with cotton blue stain and viewed under a research microscope for their morphological characteristics. The fungi specimens were identified on the basis of their morphological characteristics<sup>32</sup> and further confirmed up to the species level from the National Fungal Culture Collection of India, Agharkar Research Institute, Pune (India).

#### Protecting coating

The konex 2318, konex 2319 and zycosil-zycoprime protecting coatings were used for studies. The specific features, manufacturers and dilution ratios of water-repellent coating are presented in Table 1. The zycosil-zycoprime were utilized together as per manufacturer instruction.

#### Essential oils and determination of chemical composition

The essential oils were purchased from market: Clove oil (Qualigen, Mumbai), Peppermint oil (CDH, Delhi) and *Eucalyptus* oil (Loba Chemie, Mumbai). The essential oils were distilled using Buchi rotary vacuum evaporator under reduced pressure to check their purity. The essential oils were analysed by GC spectra (Agilent 7890 B GC with Auto sampler FID detector High Sensitivity) and GC-MS (Perkin Elmer Clarus 680 GC SQ 8 MS) from CSIR-CIMAP, Lucknow (India) to know main chemical contents.

#### Determination of zone of inhibition by essential oils

Essential oils (*Eucalyptus* oil, Peppermint oil, and Clove oil) were evaluated by using agar well diffusion method against *A. niger*, *T. purpureogenus* and



Fig. 2 — Sample collection surface of Solani aqueduct, Roorkee.

Table 1 — Details of water-repellent coatings

Coatings	Chemical base	Interaction with surface	Manufacturers	Dilution ratio
Konex 2318	Silane	Penetrating	M/s Konstruktion Chemie, Faridabaad, India	1:10 (Konex 2318 : Distilled water)
Konex 2319	Silane	Penetrating	M/s Konstruktion Chemie, Faridabaad, India	1:10 (Konex 2319 : Distilled water)
Zycosil	Silane	Penetrating	M/s Zydex Industries Pvt. Ltd, Vadodara, India	1:2:20 (Zycosil : Zycoprime : Distilled water)
Zycoprime	Acrylic	Film forming	M/s Zydex Industries Pvt. Ltd, Vadodara, India	1:2:20 (Zycosil : Zycoprime : Distilled water)



Fig. 3 — Experimental setup to determine zone of inhibition by eucalyptus, peppermint and clove oils against three fungi.

*A. alternata*<sup>33</sup>. Essential oils were diluted with 0.5% tween 20 and sterilized distilled water. Potato dextrose agar plates were inoculated with 100  $\mu$ L of standardized inoculums of *A. niger*, *T. purpureogenus* and *A. alternata* ( $1.5 \times 10^8$  CFU/mL) and spread with a sterile glass spreader. The wells of 5 mm size were made with sterile borer into agar plates containing inoculum. Different concentrations of oils (500, 1000, 1500, 2000, 4000, 6000, 8000, and 10000 ppm) of 100  $\mu$ L volume were poured into a well of pre-inoculated plates. Fluconazole was used as a positive control<sup>34</sup>. The experimental set-up for determination of zone of inhibition by essential oils is presented in Fig. 3. The plates were observed for zone of inhibition after incubation for 7 days at 25°C. The inhibitory percentage of tested essential oils on radical growth of fungal mycelium was calculated by the reported method in which per cent of inhibition =  $(dC-dT) \times 100 / dC$ ; where dC is the average diameter

of the fungal colony in control and dT is the average diameter of the fungal colony in a treatment group<sup>35</sup>.

#### Anti-fungal activity of clove oil with water-repellent coating on white cement panels

White cement test panels (150×65×5 mm) were prepared by mixing a ratio of 1:1 of white cement and sand. The 24-month-old test panels were taken for study. The panels were dried and weighed up to constant weight and divided into two equal parts. After partition, one side of the panel was labelled as UT (Untreated) and other side was labelled as T (Treated). The clove oil (10000 ppm) was prepared with a mixture of tween 20 (0.5%) in sterilized distilled water using individual repellent coating. Every specimen was coated with respective coatings according to instructions given by their manufacturer. All the panels were treated by painting brush with appropriate water-repellent coating and emulsion

of clove oil (10000 ppm). The second coat was applied after 24 h. The panels were dried up to 48 h.

The potato dextrose agar medium was prepared and autoclaved at 121°C for 30 min. The culture media was poured into each sterile petri plate (200x30 mm) and allowed to solidify. The coated panels were exposed to UV radiation for 120 seconds to sterilize them. The white cement panels were placed on centres of Petri plates over solidified medium. The experimental setup has been shown in Fig. 4. The inoculation with individual dominating fungi was done using a conidial spore suspension of  $1 \times 10^6$  spores/mL. The petri plates were incubated at  $28 \pm 0.5^\circ\text{C}$  and 85% for 28 days as per ASTM to determine the anti-fungal activity of protecting coating in triplicate<sup>17,36</sup>. The observation was recorded with the interval of 7 days up to 4 weeks.

**Effect of protective coatings on the exterior surface of Heritage Solani Aqueduct**

The field experiment was performed on vertical wall of the exterior exposed surface of heritage Solani aqueduct, Roorkee (Fig. 5). Two different sites (a and b) were selected on prolonged moistened walls for this purpose. The prepared surface area of  $300 \times 300$

$\text{mm}^2$  was treated with appropriate protecting coating (konex 2318, konex 2319, and zycosil-zycoprime) with 10000 ppm clove oil at  $25^\circ\text{C}$  by brush as per manufacture instructions and an equal area was also left untreated for comparison. In case of konex 2318, the panels were retreated with a second coat 24 h after the former. As recommended by the manufacturer, a single coat was applied for konex2319 and zycosil-zycoprime. Visual observation was recorded and field water-repellent test was conducted for up to 270 days with the interval of 30 days.

**Determination of depth of penetration of coatings**

The average depth of penetration by coatings on OPC mortar, white cement mortar and concrete specimens was determined using various colour dyes. The specimens were dried in a forced draft oven until two successive weighing after an interval of 2 h gives constant weight. Half side of the specimens were coated with water-repellent coatings and the other half were left untreated. The various dye solutions were poured using a dropper on treated and untreated parts of specimens (Fig. 6). The colours of specimens were recorded after 5 min. using various dyes, which are presented in Table 2.



Fig. 4 — Experimental set up for determination of anti-fungal potency on white cement panel.

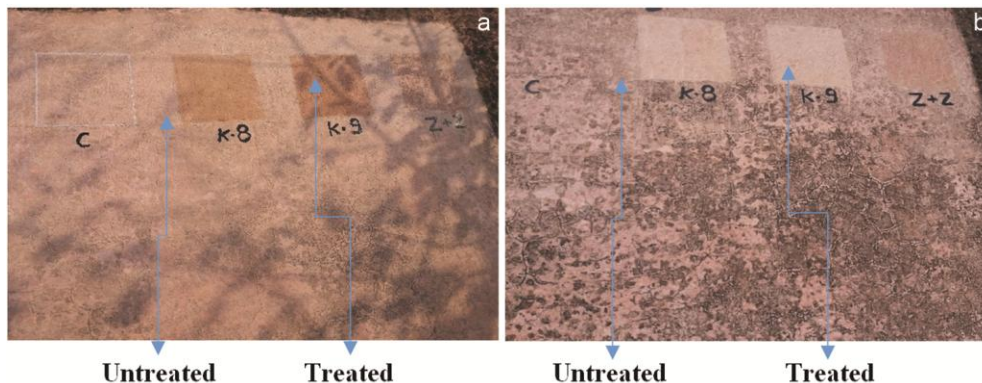


Fig. 5 — Field trial on site A of exterior surface of heritage Solani aqueduct, Roorkee: a) After treatment 0 day; and b) After treatment 270 days. C: Control; K-8: konex 2318; K-9: konex 2319 and Z+Z: zycosil-zycoprime.

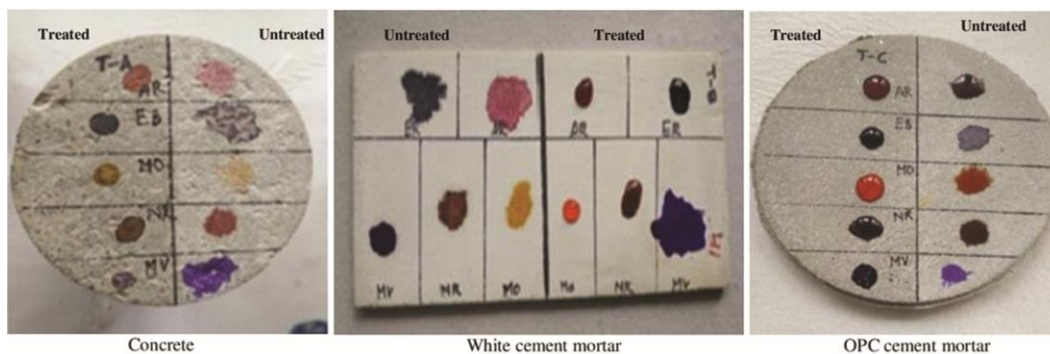


Fig. 6 — The colour variation by dyes on untreated and treated part of concrete, white cement mortar and OPC cement mortar. AR: Alizarin red; EB: Eriochrome black; MO: Methyl orange; NR: Neutral red; MV: Methyl violet.

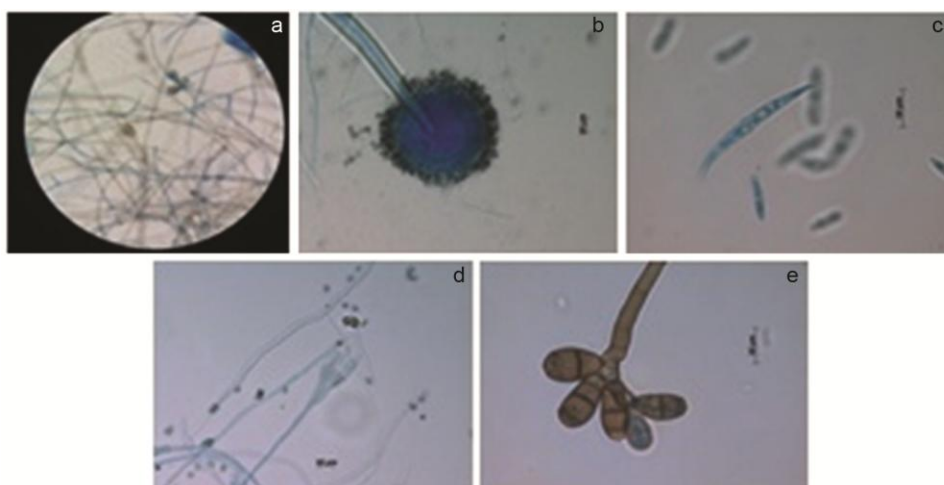


Fig. 7 — Microscopic image of identified fungi: a) *Alternaria alternata*; b) *Aspergillus niger*; c) *Fusarium equiseti*; d) *Talaromyces purpureogenus*; and e) *Curvularia pallescens*.

Table 2 — Colour chart by different dyes on various surfaces treated with silane coatings Dyes used

Sample type	Part	Alizarin red	Eriochrome black	Methyl orange	Neutral red	Methyl violet
OPC cement mortar	Treated	Brown	Shine black	Orange	Brown	Green metallic sheen
	Untreated	Pink	Faded Black	Yellow	Faded brown	Blue-violet
White Cement mortar	Treated	Brown	Black	Yellow shine	Brown	Green metallic edge
	Untreated	Pink	Faded black	Faded yellow	Faded brown	Violet
Concrete cylinder	Treated	Brown	Black	Dark yellow	Brown	Green edge
	Untreated	Pink	Faded black	Faded yellow	Faded brown	Violet

## Results

### Fungal identification

Five fungi namely; *Curvularia pallescens*, *Fusarium equiseti*, *Talaromyces purpureogenus*, *Aspergillus niger* and *Alternaria alternata* were confirmed from the National Fungal Culture Collection of India, Agharkar Research Institute, Pune (India) (Fig. 7).

### Determination of chemical composition of essential oils by GC-MS spectra

The peppermint oil contains 42.7% menthol. In the case of clove oil, the eugenol percentage was 86.95%.

The eucalyptol was found to be 78.02% in eucalyptus oil. The other main chemical compositions of these essential oils are presented in Table 3.

### Zone of inhibition of tested essential oils

The results for zone of inhibition of essential oils are presented in Table 4. It was observed that no antifungal activity was shown by Eucalyptus oil against *A. niger*, *T. purpureogenus*, and *A. alternata* in any concentration. Peppermint oil showed anti-fungal activity at 10000 ppm in following order: *Alternaria*>*Talaromyces*>*Aspergillus*. Besides clove oil showed anti-fungal activity at 2000-10000 ppm in

following order: *Alternaria*>*Talaromyces*>*Aspergillus*. The clove oil exhibited anti-fungal activity against all dominating fungi. Hence, clove oil was the better option among the other oils tested.

**Anti-fungal activity of clove oil with water-repellent coating on white cement panels**

The clove oil (10000 ppm) showed effective anti-fungal potency against all three isolated fungi when mixed with water-repellent coatings (zycosil-zycoprime, konex 2318, and konex 2319). In case of *A. niger* fungal growth was recorded 6.0, 4.7, and 2.0 mm by clove oil (1000 ppm) with coatings zycosil-zycoprime, konex 2318, and konex 2319, respectively. In case of *T. purpureogenus* fungal

growth appeared 4.5, 4.0, and 4.7 mm by clove oil (10000 ppm) with coatings zycosil-zycoprime, konex 2318, and konex 2319, respectively. However, in case of *A. alternata* fungal growth was observed 2.0, 4.0, and 5.3 mm by clove oil (1000 ppm) with coatings zycosil-zycoprime, konex 2318 and konex 2319, respectively. The growth of *A. niger*, *T. purpureogenus*, *A. alternata* was observed 9.3, 8.0, and 8.0 mm, respectively on untreated side of white cement panel. The observation for anti-fungal activity after 28 days is presented in Fig. 8.

**Effect of protective coatings on exterior surface of Solani Aqueduct**

The deterioration rating scale for all protecting coating was used for grading of degradation on field trial is shown in Table 5. The surface coating was compared for any deterioration or destruction on the treated surface due to environmental conditions such as temperature, relative humidity and colour change up to 270 days. The surface area treated by 10000 ppm clove oil with konex 2318 and konex 2319 exhibited no colour change as well as deterioration when compared with the control surface area as these are penetrating silane/siloxane-based coatings.

Table 3 — Main chemical composition of essential oils on GC/MS analysis.

Oil name	Compound	Area %
Peppermint	Limonene	0.1
	Menthone	19.5
	Iso-menthone	10.2
	Menthyl acetate	6.3
	Neo-menthol	8.3
	Menthol	42.7
	Clove oil	Methyl Salicylate
	Alfa-Copaene	0.09
	Chavicol	0.88
	Beta-Caryophyllene	8.13
	Humulene	0.39
	Eugenol	86.95
	Delta-Cadinene	0.46
Eucalyptus oil	Eucalyptol	78.02
	Beta- Linalool	0.91
	p- Cymenene	0.49
	Limonene Oxide	0.1
	Isomenthol	2.52
	Terpinen-4-01	1.75
	Alfa-Terpineol	2.52
	Carvone	0.93
	2,3-Pinenediol	1.86

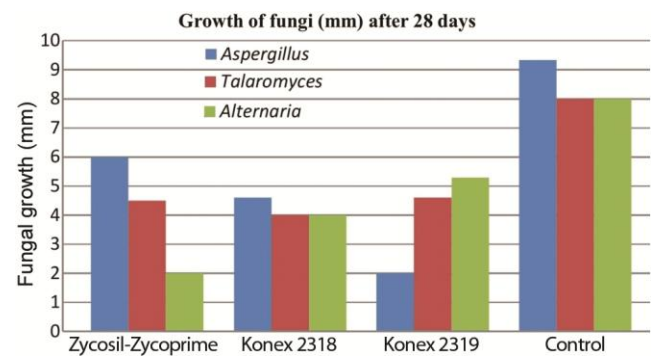


Fig. 8 — Anti-fungal activity of water repellent coatings with 10000 ppm clove oil after 28 days.

Table 4 — Antifungal activity of essential oil against *A. niger*, *T. purpureogenus*, *A. alternata*

Concentration (ppm)	Eucalyptus oil (ZOI±SE) mm			Peppermint oil (ZOI±SE) mm			Clove oil (ZOI±SE) mm		
	A	B	C	A	B	C	A	B	C
500	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1000	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1500	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.5±0.29	0.0±0.0
2000	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.2±0.3	2.5±0.29	3.3±0.3
4000	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	5.3±0.6	2.6±0.22	5.7±0.3
6000	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	10.7±0.0	3.3±0.33	6.7±0.3
8000	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	5.3±0.3	11.3±1.2	8.7±3.18	9.0±0.6
1000	0.0±0.0	0.0±0.0	0.0±0.0	1.0±0.0	4.7±0.6	8.7±0.3	12.7±0.6	13.7±2.96	14.3±3.5

(A): *Aspergillus niger*, (B): *Talaromyces purpureogenus*, (C): *Alternaria alternata*

Scale	Observation	Characteristics
0	No Deterioration	No change in appearance
1	Light deterioration	Slight change in colour
2	Moderate deterioration	Appearance of dull spots and bubbles formation
3	Heavy deterioration	Loss of glossy appearance, slight precipitation of water repellent coating and surface become dark
4	Failure	Appearance of dull spots, colour changes to dark, heavy precipitation of water repellent coating on the surface

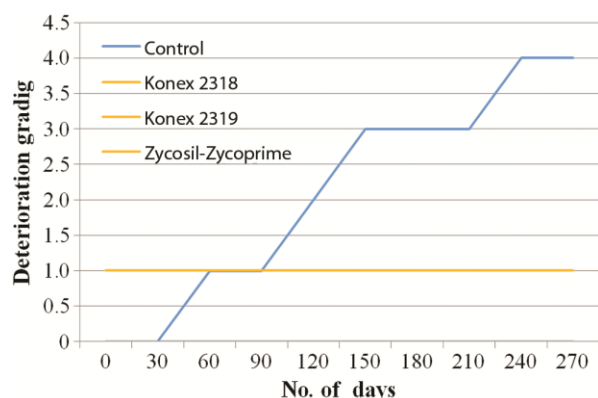


Fig. 9 — Deterioration grading on field trial at exposed surface of heritage Solani aqueduct, Roorkee. (Konex 2318, Konex 2319, Zycosil-Zycoprime exhibited deterioration grading 1. Hence, all showing same colour)

However, in case of clove oil (10000 ppm) with zycosil-zycoprime slight colour change has been observed (Table 5 and Fig. 9) as it was a mixture of silane and acrylic content. Due to surface coating, it shows a dark glossy appearance. On the basis of visual observation and the water hydrophobicity test performed, the order of effective protecting coating has been in order off konex 2319 >konex 2318 >zycosil – zycoprime (Fig. 5, 9).

#### Determination of depth of penetration of coatings

The specimen was split into two halves, perpendicular to the face on which colour dye was applied and the maximum depth of penetration of coating was measured under test area and was recorded in mm using a vernier calliper. The average depth of penetration by coatings is presented in Fig. 10. The maximum depth of penetration was recorded for konex 2319 (3.0 mm) followed by konex 2318 (2.7 mm) and zycosil-zycoprime (1.3 mm), respectively.

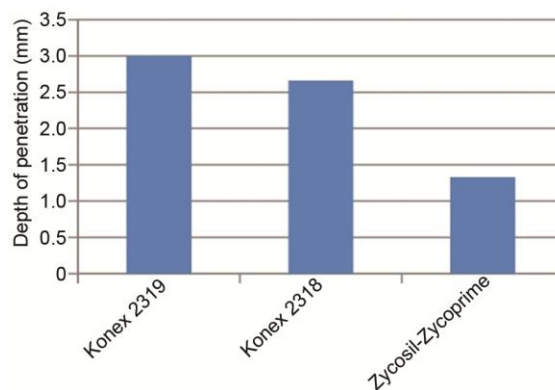


Fig. 10 — Average depth of penetration by three coatings.

#### Discussion

The synthetic fungicides are usually used to prevent fungal decay on archaeological objects. The synthetic fungicides can be highly toxic to a broad range of organisms including humans<sup>37</sup>. An attempt was made to use essential oils for searching eco-friendly alternatives to toxic chemicals which show negligible toxicity to humans. The five fungal species were identified from the heritage Solani Aqueduct, Roorkee: *C. pallescens*, *F. equiseti*, *T. purpureogenus*, *A. niger*, and *A. alternata*. The results indicate that most of prevailing fungal species on sample site are common building fungi. This finding is in agreement with Gupta and Sharma, reported that *A. niger*, *Cladosporium oxysporum*, *Curvularia lunata*, *C. clavata*, *Fusarium sp.*, *Mucor sp.*, *Mycelia sterilia (white)*, *Paecilomyces variotii*, *P. chrysogenum*, *Penicillium sp.*, *Trichoderma viride* from Sita Devi temple, Deorbija, Chhattisgarh India<sup>38</sup>. The three essential oils were evaluated against three dominating fungi to determine the zone of inhibition. It was observed that no antifungal activity was shown by Eucalyptus oil against *A. niger*, *T. purpureogenus* and *A. alternata* in any concentration. The result is in agreement with Elaissi *et al.*, as they studied the antifungal activity of different varieties of eucalyptus oil and reported variable potency as the antifungal activity of eucalyptus essential oils altered remarkably with the species of eucalyptus plant and within strains of fungi<sup>39</sup>. The peppermint oil showed moderate antifungal activity against *A. alternata* (8000-10000 ppm) and *T. purpureogenus* (10000 ppm). The result is similar to finding of Saharkhiz *et al.*<sup>14</sup>, as they reported that variation in anti-fungal activity can be occurred by a change in the composition of essential oils. Alpha terpinenol and menthol concentration in composition of essential oils might be varied

by development stage of plant and change in geographical location from which plants were collected<sup>14</sup>. Out of three essential oils, clove exhibited significant anti-fungal activity against *T. purpureogenus*, *A. niger*, *A. alternata* at 10000 ppm concentration. The clove oil (10000 ppm) also showed better anti-fungal potency with all three water-repellent coatings namely konex 2318, konex 2319 and zycosil-zycoprime. The result of present study confirmed antifungal potency of clove oil with water-repellent coating for inhibition of fungi on cement panels for up to 28 days in laboratory and as well as on field trial at Solani Aqueduct, Roorkee. The antifungal activities of clove oil mainly depend on the eugenol concentration, which was determined 86.95% on GC-MS spectra. A similar antifungal result is reported<sup>40</sup>. The findings of the present study would be useful to search for new eco-friendly alternative anti-fungal coating, which may be applied to heritage structures without changing aesthetic appearance. An eco-friendly anti-fungal chemical with a suitable coating would be the possible solution to avoid fungal colonization on heritage structures.

### Conclusion

A large number of monuments and historic buildings spread all over the world constitute one of the finest examples of mankind's cultural heritage. The Solani Aqueduct is 175 years old heritage structure in Roorkee, India. The exterior surface of structure is deteriorated due to fungal infestation. Five fungi namely; *C. pallescens*, *F. equiseti*, *T. purpureogenus*, *A. niger*, *A. alternata* were isolated and identified from its surface. Out of three, the clove oil (10000 ppm) exhibited potential antifungal activity against all three dominating fungi (*T. purpureogenus*, *A. niger*, *A. alternata*) tested on minimum inhibitory concentration evaluation on petriplates as well as when mixed with protecting coatings (konex 2319 >konex 2318 >zycosil-zycoprime). The maximum depth of penetration was recorded for konex 2319 (3.0 mm). On the basis of visual observation and water hydrophobicity test performed on field trial at the surface of heritage solani aqueduct, Roorkee, the order of effective protecting coating with clove oil (10000 ppm) has been in the order off konex 2319 >konex 2318 >zycosil-zycoprime, respectively. The combination of the antifungal potency of essential oils, their fungi toxicity with water-repellent coatings, the average thickness of penetration and the effect of protecting coating on the exterior surface of Solani

Aqueduct, Roorkee has allowed us to understand and compare the performance of different coating on artificial ageing.

### Acknowledgement

The authors acknowledge the funding from the Department of Science and Technology, Government of India through the project on "Creation of center of excellence on Indian heritage structures (DST/TDT/SHRI(WG)-05/2020" dated 16.12.2020.

### Conflict of interest

The authors declare no conflict of interest.

### References

- Geweely N S, Afifi H A, Ibrahim D M and Soliman M M, Efficacy of essential oils on fungi isolated from archaeological objects in Saqqara excavation, Egypt, *Geomicrobiol*, 2019, **3**, 148-168.
- Singh J, Fungal problems in historic buildings, *J Arch Conserv*, 2000, **6**, 17-37.
- Sterflinger K, Fungi: Their role in deterioration of cultural heritage, *Fungal Biol Riv*, 2010, **24**, 47-55.
- Macwan S R, Dabhi B K, Aparnathi K D and Prajapati J B, Essential oils of herbs and spices: Their antimicrobial activity and application in preservation of foods, *Int J Curr Microbiol Appl Sci*, 2016, **5**, 885-901.
- Tongnuanchan P and Benjakul S, Essential oils: Extraction, bioactivities, and their uses for food preservation, *J Food Sci*, 2014, **79**, 1231-1249.
- Bakkali F, Averbeck S, Averbeck D and Idaomar M, Biological effects of essential oils—A review, *Food Chem Toxicol*, 2008, **46**, 446-475.
- Božović M, Garzoli S, Sabatino M, Pepi F, Baldissarroto A, *et al.*, Essential oil extraction, chemical analysis and anti-Candida activity of *Calaminthanepeta* (L.) Savi subsp. glandulosa (Req.) Ball-New Approaches, *Molecules*, 2017, **22**(2), 203.
- Hu Y, Zhang J, Kong W, Zhao G and Yang M, Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*, *Food Chem*, 2007, **220**, 1-8.
- Prakash B, Singh P, Kedia A and Dubey N K, Assessment of some essential oils as food preservatives based on antifungal, anti-aflatoxin, antioxidant, activities and *in vivo* efficacy in food system, *Food Res Int*, 2012, **49**, 201-208.
- Ghalem B R, Essential oils as antimicrobial agents against some important plant pathogenic bacteria and fungi. In: *Plant-Microbe Interaction: An Approach to Sustainable Agriculture*; Choudari, edited by D K Varma, A Tuteja N, (Springer Nature Singapore Pvt. Ltd.: Singapore), 2016, 271-296.
- Lang G and Buchbauer G, A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungal, *Flavour Fragr J*, 2012, **27**(1), 13-39.
- Zhou L, Li F, Huang L, Yuan and Bai L, Antifungal activity of Eucalyptus oil against rice blast fungi and the possible mechanism of gene expression pattern, *Molecules*, 2016, **21**, 621. doi:10.3390/molecules21050621.

- 13 Moghaddam M, Pourbaige M, Tabar H K, Farhadi N and Hosseini M A, Composition and antifungal activity of peppermint (*Mentha piperita*) essential oil from Iran, *J Essent Oil Bear Plants*, 2013, **16**(4), 506-512.
- 14 Edris A E and Farrag E S, Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase, *Food/Nahrung*, 2003, **47**(2), 117-121.
- 15 Saharkhiz M J, Motamedi M, Zomorodian K, Pakshir K, Miri R, *et al.*, Chemical composition, antifungal and anti-biofilm activities of essential oil of *Mentha piperita* L., *Int Sch Res Network*, 2012, **2012**, doi, 10.5402/2012/718645.
- 16 Verma R K, Chaurasia L and Katiyar S, Evaluation of antifungal potency of *Citrus* essential oils against building fungi, *Pestology*, 2007, **31**, 29-31.
- 17 Verma R K, Chaurasia L and Katiyar S, Potential antifungal plants for controlling building fungi, *Nat Prod Rad*, 2008, **7**, 374-387.
- 18 Singh T and Chittenden C, Efficacy of essential oil extracts in inhibiting mould growth on panel products, *Build Environ*, 2010, **45**(10), 2336-2342.
- 19 Verma R K, Chaurasia L and Kumar M, Anti-fungal activity of essential oils against selected building fungi, *Indian J Nat Prod Res*, 2011, **2**(4), 448-451.
- 20 Kizil S, Hasimi N, Tolan V, Kilinc E and Uksel U Y, Mineral content, essential oil components and biological activity of two mentha species (*M. piperita* L., *M. spicata* L.), *Turk J Field Crops*, 2010, **15**(2), 148-153.
- 21 Tyagi A K and Malik A, Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: Microscopic observations and chemical characterization of cymbopogon citrates, *BMC Complement Alternat Med*, 2010, **10**(1), 1-11.
- 22 Behnam S, Farzaneh M, Ahmadzadeh M and Tehrani A S, Composition and antifungal activity of essential oils of *Mentha piperita* and *Lavendula angustifolia* on post-harvest phytopathogens, *Commun Agric Appl Biol Sci*, 2006, **71**, 1321-1326.
- 23 Khaledi N, Taheri P and Tarighi S, Antifungal activity of various essential oils against *Rhizoctonia solani* and *Macrophomina phaseolina* as major been pathogen, *J Appl Microbiol*, 2014, **118**(3), 704-717.
- 24 Kotterachchi N S, Sammani A, Kelaniyangoda D B and Samarasekara R, Antifungal activity of essential oils of ceylon Eucalyptus species for the control of *Fusarium solani* and *Sclerotium rolfsii*, *Arch Phytopathol Plant Prot*, 2012, **45**(17), 2026-2035.
- 25 Patel R M and Jasrai Y T, Antifungal potency of Eucalyptus globules essential oil against important plant pathogenic fungi, *CIB Tech J Microbiol*, 2015, **4**(1), 42-52.
- 26 Mahato R, Awasthi S, Kumar S and Bhatnagar T, A comparative study of effect of essential oil and alcoholic extract of Eucalyptus leaves on medically important bacterial and fungal isolates, *Int J Curr Microbiol Appl Sci*, 2015, **4**(3), 504-508.
- 27 Tyagi P, Verma R K and Jain N, Fungal degradation of cultural heritage monuments and management options, *Curr Sci*, 2021, **121**(12), 1553-1560.
- 28 Felicia W X L, Rovina K, Vonnice J M, Aqilah M N N, Erna K H, *et al.*, Consolidating plant based essential oils onto polysaccharides based coatings: Effect on mechanism and reducing postharvest losses of fruits, *Appl Food Res*, 2022, **2**(2), 1000226.
- 29 Verma R K and Chourasia A, Protection of bio-deteriorated reinforced concrete using concrete sealers, *Int J Mater Chem Phys*, 2015, **1**, 11-19.
- 30 Verma R K and Devi D, Studies for antifungal activity of selected concrete sealers on white cement panels, *Int J Sci Eng Technol Res*, 2015, **4**(6), 2081-2087.
- 31 Molina M T, Cano E, Leal J, Fort R, Buerger M A, *et al.*, Protective coatings for metals in scientific technical heritage: The collection of the Spanish national museum of science and technology, *Heritage*, 2023, **6**(3), 2473-2488.
- 32 Singh R, Upadhyay S K, Sharma I, Kamboj P, Rani A, *et al.*, Assessment of enzymatic potential of soil fungi to improve soil quality and fertility, *Asian J Biol Life Sci*, 2020, **9**(2), 163-168.
- 33 Balouiri M, Sadiki M and Ibsouda S K, Methods for *in vitro* evaluating antimicrobial activity: A review, *J Pharm Anal*, 2016, **6**(2), 71-79.
- 34 Seleem D, Benso B, Noguti J, Pardi V and Murata R M, *In vitro* and *in vivo* antifungal activity of lichochalcone – A against *Candida albicans* biofilms, *Plos One*, 2016, **11**(6), e0157188.
- 35 Xa L T and Nghia N K, Antagonistic activity against plant pathogenic fungus by various indigenous microorganism from different cropping systems in Soc Trang Province, *Environ Nat Res J*, 2020, **18**(3), 249-256.
- 36 ASTM G21-15: Standard practice for determining resistance of synthetic polymeric materials to fungi, 2021, 1-6.
- 37 Zubrod J P, Bundschuh M, Arts G, Bruhl A, Imfeld G, *et al.*, Fungicides: An over looked pesticides class?, *Environ Sci Technol*, 2019, **53**(7), 3347-3365.
- 38 Gupta S P and Sharma K, Bio deterioration and preservation of sitadevi temple, Deorbija, Chhattisgarh India, *Int J Conserv Sci*, 2011, **2**(2), 89-94.
- 39 Elaissi A, Rouis Z, Salem N A, Mabrouk S, ben Salem Y, *et al.*, Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities, *BMC Complement Alternat Med*, 2012, **12**, 1-15.
- 40 Alma M H, Ertas M, Nitz S and Kollmannsberger H, Chemical composition and content of essential oil from the bud of cultivated Turkish clove (*Syzygium aromaticum* L.), *Bio Resour*, 2007, **2**(2), 265-269.