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

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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 of clove oil (10000 ppm) with konex 2319 > konex 2318 > zycosil-zycoprime.

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

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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. 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. 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.

Essential oils are usually complex mixtures of natural compounds. These are used for their antimicrobial activity in food products. They can represent one of the most promising natural products for fungi control. The essential oils obtained from different plants or herbs exhibited intense antifungal properties. Eucalyptus and peppermint oils were found effective against plant pathogenic fungi. Menthol was found to be an individual aroma responsible for the antifungal activity of peppermint essential oil. The peppermint essential oil exhibited strong antifungal activities against examined fungi at concentrations ranging from 0.12 to 8.0 μL/mL. Anti-fungal activities of essential oil and methanolic extracts of peppermint were demonstrated. The
essential oils from three *Eucalyptus* species showed significant antifungal activity against *Sclerotium rolfsii* and *Fusarium solani*. The essential oil of *Eucalyptus* possesses good anti-fungal potential against a few fungal strains. Antifungal activity and minimum inhibitory concentration (MIC) of tested essential oils with different concentrations (0.125, 0.25, 0.5, 0.75, 1 µL/mL) were determined. The potency of various essential oils against fungal growth on cultural heritage was reviewed. The main phytochemical composition of eucalyptus, peppermint and clove oils are summarized.

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. 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 stolinifer*, *Penicillium chrysogenum*, and *Mucor racemosus* up to 28 days. Transparency and stability of the visual aspect of coatings are very important factors to fulfil heritage conservation criteria.

Commercially available water-repellent coatings are based on silicon, silane, silicate, silicone, 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. 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 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.
Essential oils were diluted with 0.5% tween 20 and sterilized distilled water. Potato dextrose agar plates were inoculated with 100 µL of standardized inoculums of \( A. \) niger, \( T. \) purpureogenus and \( A. \) alternata \( (1.5 \times 10^8 \text{ CFU/mL}) \) 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 µL volume were poured into a well of pre-inoculated plates. Fluconazole was used as a positive control. 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 radial growth of fungal mycelium was calculated by the reported method in which per cent of inhibition = \((dC-dT)\times100/Dc\); 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.

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

White cement test panels \((150\times65\times5 \text{ mm})\) 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 1x10^6 spores/mL. The petri plates were incubated at 28±0.5°C and 85% for 28 days as per ASTM to determine the anti-fungal activity of protecting coating in triplicate. 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 × 300 mm^2 was treated with appropriate protecting coating (konex 2318, konex 2319, and zycosil-zycoprime) with 10000 ppm clove oil at 25°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.
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 antifungal 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. The growth 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 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.

<table>
<thead>
<tr>
<th>Oil name</th>
<th>Compound</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peppermint</td>
<td>Limonene</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Menthone</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>Iso-menthone</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>Menthyl acetate</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>Neo-menthol</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Menthol</td>
<td>42.7</td>
</tr>
<tr>
<td>Clove oil</td>
<td>Methyl Salicylate</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Alfa-Copaene</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Chavicol</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Beta-Caryophyllene</td>
<td>8.13</td>
</tr>
<tr>
<td></td>
<td>Humulene</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Eugenol</td>
<td>86.95</td>
</tr>
<tr>
<td></td>
<td>Delta-Cadinene</td>
<td>0.46</td>
</tr>
<tr>
<td>Eucalyptus oil</td>
<td>Eucalyptol</td>
<td>78.02</td>
</tr>
<tr>
<td></td>
<td>Beta- Linalool</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>p- Cymenene</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Limonene Oxide</td>
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<tr>
<td></td>
<td>Isomenthol</td>
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<tr>
<td></td>
<td>Terpinen-4-01</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>Alfa-Terpineol</td>
<td>2.52</td>
</tr>
<tr>
<td></td>
<td>Carvone</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>2,3-Pinanediol</td>
<td>1.86</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Eucalyptus oil (ZOI±SE) mm</th>
<th>Peppermint oil (ZOI±SE) mm</th>
<th>Clove oil (ZOI±SE) mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>500</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>1000</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>1500</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>2000</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>4000</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>6000</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>8000</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>1000</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

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

### Table 5 — Deterioration rating scale for field trial

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

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. 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), *Paeclomyces variotii*, *P. chrysogenum*, *Penicillium sp.*, *Trichoderma viride* from Sita Devi temple, Deorbija, Chhattisgarh India. 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. 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., 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\(^{14}\). 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\(^{40}\). 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.

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**Conflict of interest**

The authors declare no conflict of interest.

**References**


