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FUMIGATION OF EUCALYPTUS OIL FOR CONTROLLING STRONG ROOM FUNGI AT JORASANKO MUSEUM (TAGORE'S RESIDENCE), INDIA: A STUDY FOR SUSTAINABLE CONSERVATION

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Abstract

Presently thymol is used as fungal repellent in several museums worldwide. Thymol is a reported decolorizing (foxing) agent and also harmful for human health (toxicity category-III). In the present study it has been observed that thymol is being used to conserve about three thousand rare documents belonging to Nobel Laureate poet Ranindranath Tagore and his ancestrals in Jorasanko Museum, India. The objective of our study was to promote a suitable nontoxic alternative for long term conservation of museum materials. Eucalyptus oil was selected for this purpose. Percentage Mycelial Inhibition (PMI) had been studied using 24 ppm, 48 ppm, 72 ppm and 96 ppm of eucalyptus oil on ten fungi isolated from the strong room of the museum. Both thymol and eucalyptus oil had been fumigated and fungal counts were observed after two days. Principal component analysis (PCA) revealed that 48 ppm eucalyptus oil and 4 days fumigation frequency yield optimum fungal control. It was sensitive for individual strains like Aspergillus tamari (32% removal) and Trichoderma sp (64% removal) which was not controlled by thymol fumigation. This study revealed that eucalyptus oil has better potentiality and can be used for long-term conservation of museum objects in future.

Keywords: *Eucalyptus oil; Museum conservation; Museum fungi; Foxing.*

Introduction

Museum, libraries and archives sometimes have suitable conditions for fungal growth. Fungal spores get entry into indoor atmosphere through wind currents and settle on various objects by impaction [1, 2]. These spores are disseminated by the action of wind and cause severe losses of valuable historical and cultural documents. Tropical countries, where high humidity and temperature are frequent, have environmental conditions that enhance the development of these microorganisms. This situation can bring risks to biodeterioration by fungi in books and documents, causing the decay of these antique and rare publications [3]. Fungi can hydrolyze a wide variety of polymers, including cellulose, as a result of their efficient enzymes [4]. Cellulolytic fungi, which use cellulose as substratum, when growing in favorable environmental conditions, can destroy paper material in a short time [5]. The old textile materials, leather, lime stone photograph and oil paintings are also vulnerable in fungal deterioration in museum [6-9]. *Aspergillus, Alternaria, Aspergillus, Cladosporium*, and

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Penicililum are very common in museum environment throughout the world [10]. Another recent investigation showed that *Sarcinomyces sp, Pithomyces sp, Scolecobasidium sp*. were also found in the degraded stone monuments [11].

Several techniques have been developed for book and document conservation in order to reduce the threat of biodeteriorating agents, such as fungi. Some of these techniques involve the use of very toxic and expensive chemicals, including ethylene oxide, which has carcinogenic properties and is banned in a number of countries [12, 13]. An alternative is the use of botanical fungicides, a promising treatment in the preservation field. In past thymol fumigation chamber has been widely used for eliminating fungi, in long run this compound were not used due to health hazards of museum staff and deleterious impacts of archival materials. These chemical not only control fungi but also increase the drug resistant fungal strains in environment [14]. Excessive use of chemical fungicides has lead to increased environmental pollution, harmful effects on human health and resistance among fungi to the used chemicals. Several red flags have been raised by the conservators and curators about low cost, environment friendly method for treating low to moderate quantity of deterioration in museum exhibit [15]. Essential oils of various plants species are now being used in the pharmaceutical, toiletries, cosmetics, and food industries as they have broad applications regarding the antiseptic, antihyperglycemic, anti-inflammatory, flavoring, and antioxidant properties [16]. These oils are extracted from the natural plant products and they are mostly nonpersistent biodegradable compounds [17]. Eucalyptus oil is particularly useful as it possesses a wide range of desirable properties for both agriculture and non-agriculture pest management. The antimicrobial activities of eucalyptus oils have been well known and are being used in flavoring, pharmaceutical, toiletries and food preservative industry. At ancient time different natural products cedar wood oils, camphor, clove, eucalyptus leaves and musk had been used to maintain the papyrus record. Apart from all these, turmeric, black cumin, *Neem* leaves, black piper, *Ajoaine, Snakekin, kapur, Aswagandha* leaf, tobacco leaf, flower of *gutidabadi*, *kumkum* fruit etc. are also used against insects and micro-organisms [18].

The present research aims to eliminate the fungal contamination from the strong room of the Jorasanko Museum (Tagore's Residence) applying environment friendly chemicals. Here we selected eucalyptus oil against the isolated fungal strains of the study area with the aim that our results may help to evaluate the removal potentiality existing antifungal agent thymol and experimental chemical, the eucalyptus oil.

The Tagore Residence-Study area

The Tagore's residence at Jorasanko, North of Kolkata, West Bengal, India, is the ancestral home of the Tagore family. It is currently located on the Rabindra Bharati University campus at 6/4 Dwarakanath Tagore Lane Jorasanko, Kolkata 700007. It is the house in which the poet and first non-European Nobel laureate, Rabindra Nath Tagore was born. Our study site Jorasanko Tagore's House is two hundred years old (built in the 18th century) and is situated near the Hooghly River at 20 ft above sea level. The climate is warm and humid throughout the year. The temperature fluctuates between 24°C and 38°C during summer and 12–27°C in winter. Average rainfall is about 1,582mm per annum (June–September). The house has been restored to reflect the way the household looked when the Tagore family lived in it and currently serves as the Tagore museum for Kolkata. The museum exhibits details about the history of the Tagore family including their involvement with the Bengal Renaissance. This historic monuments and buildings represent a National cultural heritage, constitutes a high societal priority. Future generations must have the opportunity to witness former social structures, the endeavor, aesthetic sentiments and architectural achievements of their ancestors. The building is now used as a well visited museum, conserving 3746 types of archival documents (Fig. 1).

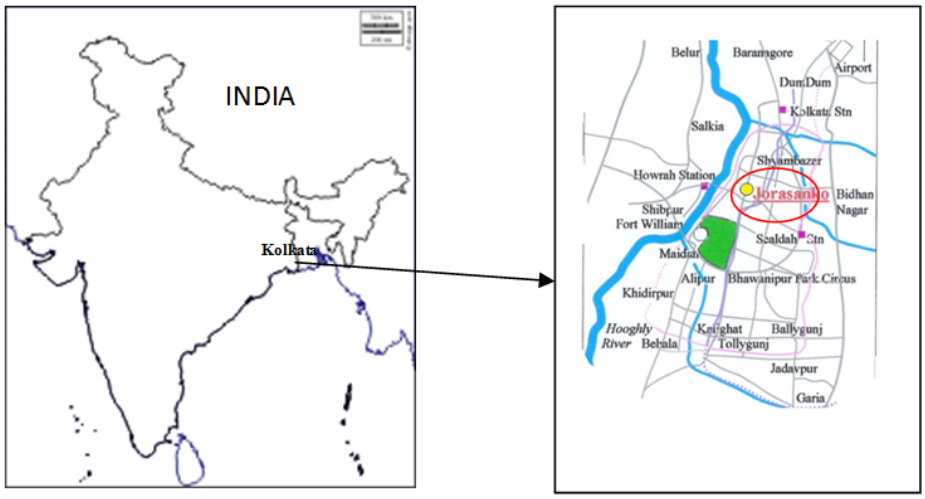

Fig. 1. Location map of study area

Materials and Method

Isolation of Fungi from the Strong Room. Air sampling was carried out for a period of one year from April 2014 to May 2015. Concerning indoor air samples, air was collected once a week, at 1 pm in the afternoon in the same place on the site of the study building.

The Andersen two-stage viable (microbial) particle sampler (2-STG) has been developed for monitoring bioaerosols. It is a multi-orifice, cascade impactor with 400 holes per stage, drawing air at a flow rate of 28.3L·min^{-1}. The different stages separate the airborne particles in size fractions. For this study air sampling was done on Potato Dextrose Agar Medium (HiMedia, Mumbai, India) at one meter height from the ground. The sampler was operated for three minutes at the site in duplicate and an average was taken. Colony forming unit per cubic meter (cfu/m^3) is calculated from the following equation:

Colony Forming Unit (cfu) = $\frac{1000P}{RT}$ cfu/m^3

Where P is the number of colonies counted on the sample plate after correction by using positive hole conversion table provided by Andersen(1958), T is the duration and R is the air-sampling rate in L·min^{-1} (28.3 L·min^{-1}).Fungal species were identified on the basis of their morphological characteristics when the development of the colonies was sufficient.

Percentage Mycelial Inhibition (PMI) Test for Eucalyptus oil. The test plates were prepared by seeding 100µL of spore suspension having 100±20 numbers of spores of each isolated strain into molten agar plate. Well diffusion method had been used and 24ppm, 48ppm, 72ppm and 96ppm doses of eucalyptus oil were added in the well. The plates were incubated at 30^{o}C and observation was taken after 2 to 21 days. The 60% essential eucalyptus oil was purchased from SRL chemicals (Code No 93050).

The diameter of the inhibitory zone will be measured and calculated accordingly.

Percentage Mycelial Inhibition (PMI)**,** I = [(*C*-*T*)/*C*] /100

Where I is inhibition (%), *C* is the diameter (mm) of the well on the control Petri plate, and *T* is the inhibitory zone diameter (mm) of the mycelium on the test Petri plate.

In-vitro fumigation of Thymol and Eucalyptus oil **.**The spore of each of the fungal species was inoculated in a culture plate having 58cm^2 surface areas. The inoculation loads were 100±20 of each plate. Thymol (30ppm) and Eucalyptus oil (48ppm) has also been fumigated at the middle of the culture plate. The whole experimental set up was kept in the incubator at 30^{o}C for 2 days. This eucalyptus oil dose has been selected by using the result

obtaining from PCA of the Percentage Mycelial Inhibition (PMI) Test for eucalyptus oil and the 30ppm thymol dose were being fumigated in the strong room of the *Jorasanko* Museum regularly.

Results and Discussions

Fungal loads of strong room and identification of fungi. In the study carried out with the aid of the Anderson two stage air samplers, a total of 10 types of cultivable fungal species were identified. 335±26cfu/m^3 spore load were found before treating with thymol at monsoon. As regard to the identification of isolated fungi of the store room, the highest load found in all was *Penicillium oxalicum* (156±4) followed by *Paecilomyces variotii* (120±5), *Aspergillus tamari* (118±4), *Aspergillus niger* (106±6), *Aspergillus ochraceous* (89±6), *Aspergillus fumigatus* (46±7), *Fusarium semitectum* (27±6), *Curvularia lunata* (25±6), *Trichoderma sp* (25±6) and *Curvularia eragrostidis* (23±5), as seen in figure 2. Investigator reported that near about 105 items (91.3%) in an indoor environment of a museum were positive for the presence of fungi. The most common isolated fungi were *Aspergillus* spp. (32.9%) followed by *Penicillium* spp. (19.1%), and *Madurella* spp. (5.2%).

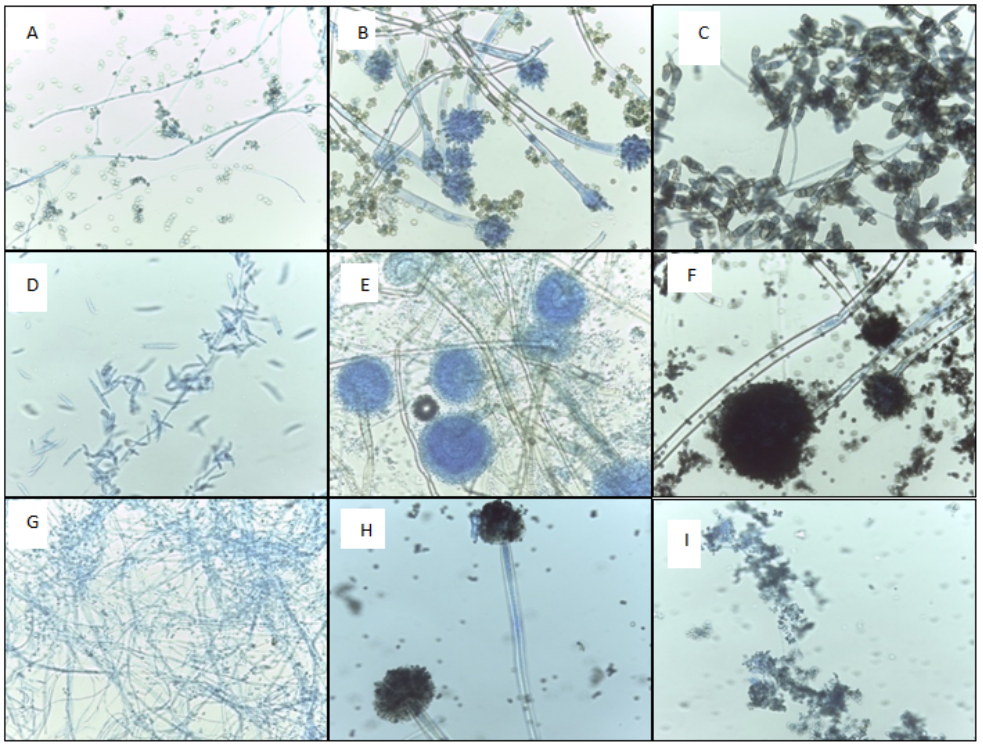

Fig. 2. Isolated fungal species from the strong room of the Jorasanko Museum:
A - *Penicillium oxalicum* RBU1, B - *Aspergillus tamarii* RBU2,C - *Fusarium semitectum* RBU3,
D - *Curvularia lunata* RBU4,E - *Aspergillus ochraceous* RBU5, F - *Aspergillus niger* RBU6,
G - *Paecilomyces variotii* RBU7, H - *Aspergillus fumigates* RBU8, I - *Trichoderma sp.* RBU9)
(Olympus CH20i Model CH20iBIMF and Digity microscopic camera Model No 210 Dewinter Caliper Pro)

Another work revealed that *Botryodiplodia theobromae, Trichoderma longibrachiatum, Aspergillus candidus, Aspergillus ustus* and *Aspergillus terreus* were found in sycamore wood blocks of ancient coffin present in Egyptian museums [19]. Thirteen species of fungi were also

obtained from various places of stone structures of an Indian Shiva Temple in which *Aspergillus fumigatus* Fr. species are found in all samples and their percentage frequency is very high [20]. Like our study, Pangallo et al also isolated mycoflora on the wooden art objects of the museum environment [21]. The art objects were principally colonized by fungi. The most commonly isolated strains were represented by hyphomycetes of the genera *Penicillium, Aspergillus, Cladosporium* and *Chaetomium*. A very recent work reported the fungal contamination in the work environment of a Polish Museum [2].

Evaluating Thymol fumigation practices. The removal efficiency of the thymol was not the same among the ten isolated fungi of the strong room (Fig. 3). After fumigation of thymol the loads of *Fusarium semitectum*, *Aspergillus niger*, *Aspergillus ochraceous*, *Curvularia eragrostidis* and *Trichoderma sp.* were 23±2cfu/m^3, 100±6cfu/m^3, 82±5cfu/m^3, 20±3cfu/m^3 and 23±5cfu/m^3 respectively, where as other five isolates removed better after 48 hours of the fumigation of thymol in the strong room (Fig. 3). They were *Penicillium oxalicum* (150 ±12cfu/m^3), *Paecilomyces variotii* (105±10cfu/m^3), *Aspergillus tamari* (80±12cfu/m^3) and *Curvularia lunata* (22±6cfu/m^3). Thymol, 5-methyl-2-isopropyl-1-phenolit is a colorless, translucent crystalline flake having low vapor pressure, a property which keeps it from being effective in an open system and from having any residual fungicidal effect on objects which have been treated.

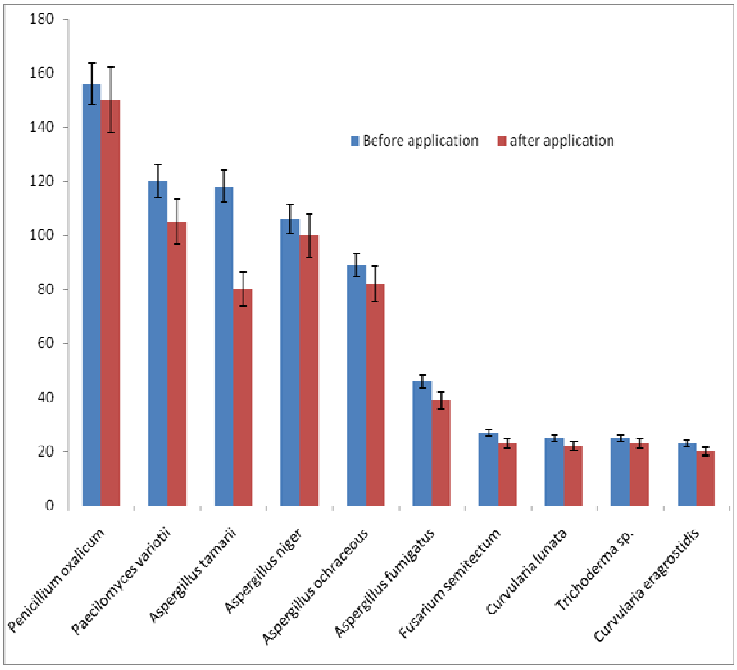

Fig. 3. Loads of isolated fungi before and after fumigating thymol (30ppm) in museum strong room

In fumigation chamber, thymol can effectively kill active mold growing on a damp surface [22]. Here we also fumigate thymol in the close cupboards of the museum strong room and it also removed fungi effectively. In the Jorasanko museum last 25 years the thymol fumigation method had been used. Long term use of same chemical sometime creates resistant strain. An Antifungal drug susceptibility test revealed that some pathological fungi were resistant to the common antifungal agents like caspofungin, posaconazole and voriconazole like compounds [23]. It is established that thymol was an efficient antifungal agent even among the clinical isolated [24]. In the present study Thymol removed *Aspergillus tamari, Penicillium*

oxalicum, Aspergillus fumigates, Curvularia lunata, Trichoderma sp., Curvularia eragrostidis, Aspergillus ochraceous, Aspergillus niger and *Fusarium semitectum* upto 16%, 15%, 13%, 11%, 10%, 6%, 5%, 3% and 3% respectively (Fig. 3) which were not enough for conserving strong room objects of the museum. The fungal residue might degrade the available objects in the strong room. Despite the fungal removal by thymol, an earlier study reported that the thymol fumigation decolorized of printing materials and they became yellowish in colour [25]. The present study was not aimed to observe the deterioration status of the museum strong room and we rather studied the application potentiality of new alternative antifungal agent like eucalyptus oil.

Percentage Mycelia Inhibition (PMI) by eucalyptus oil and evaluating the effect of Eucalyptus oil. We used eucalyptus oil for each isolated fungi from the strong room. The highest PMI was observed among *Aspergillus fumigates* (88.46), followed by *Aspergillus ochraceous* (82.6), *Trichoderma sp.* (75), *Aspergillus niger* (66.66), *Curvularia lunata* (66.66), *Penicillium oxalicum* (60.71), *Paecilomyces variotii* (60), *Curvularia eragrostidis* (59.32) and *Aspergillus tamari* (22.72) after two days of eucalyptus oil application. After four days of incubation period the highest PMI was observed among *Aspergillus fumigates* (78.94) followed by *Aspergillus ochraceous* (75), *Trichoderma sp.*(61.36), *Aspergillus niger* (57.14), *Curvularia lunata* (54.28), *Fusarium semitectum* (48.14), *Penicillium oxalicum* (42.85), *Paecilomyces variotii* (41.66), *Curvularia eragrostidis* (37.5) and *Aspergillus tamari*. After six days of incubation the applied eucalyptus oil could not inhibit the growth of the isolated fungi. The mycelia of fungi grew simultaneously as they grew in the control plates. It shows either zero or negative results in PMI calculation. Mycelia growth is negatively correlated (R^2 = 0.5 and $p > 0.5$) with the incubation period and positively correlated (R^2 = 0.4 and $p > 0.5$) with the eucalyptus oil concentration.

The goals of the particular study were to quantify the dose and time required for maximum removal of the fungi from the cupboard of the strong room. Here we applied commercial eucalyptus oil on the isolated fungal strains. After fumigation of eucalyptus oil the growth of mycelia was restricted. The Percentage Mycelial Inhibition (PMI) data revealed that the maximum inhibition was observed in *Aspergillus fumigatus* after day 2. The eucalyptus dose was 96ppm/100 numbers of fungal spores (Fig. 4).

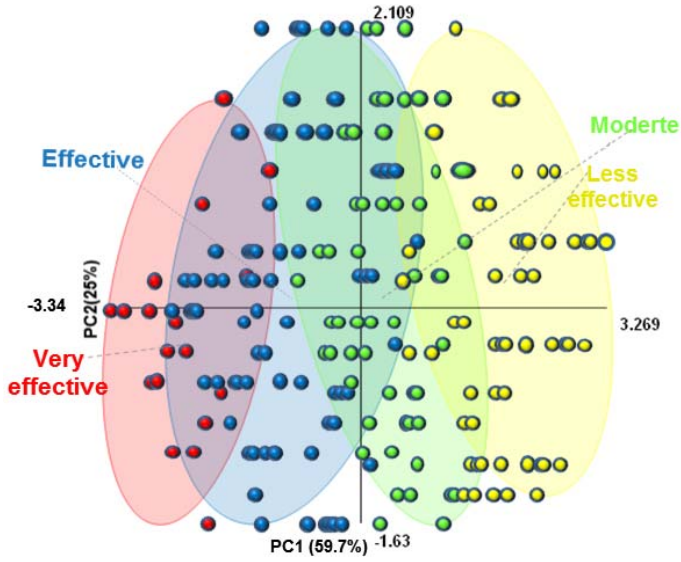

Fig. 4. PCA loadings plot on the three factors (dose of eucalyptus oil, PMI and incubation time) of isolated fungal species SPC 2

The PMI increased as the dose of oil increased ($R^2 = 0.68$ and $p > 0.5$), the PMI was inversely correlated with the day ($R^2 = 0.8$ and $p > 0.5$). After fumigation of 24ppm of eucalyptus oil among 100 numbers of fungal spores of ten isolated strains, *Aspergillus fumigates* had showed the highest PMI followed by *Aspergillus ochraceous , Penicillium oxalicum, Fusarium semitectum, Curvularia lunata, Aspergillus niger ,Trichoderma sp., Curvularia eragrostidis, Paecilomyces variotii* and *Aspergillus tamari*. After 4 days to 21 day the inhibitory effects of eucalyptus decreased and the re-growth of mycelia was observed. The mycelia of *Penicillium oxalicum, Curvularia lunata and Curvularia eragrostidis* started growing again after the 4th days of fumigation. The action of eucalyptus oil remained effective against the species *Aspergillus fumigatus* and *Aspergillus ochraceous* (Table 1).

Table 1. Fumigation of 24ppm of eucalyptus oil per 100±20 spore of each isolate

Species	2 day	4 day	6 day	14 day	21 day
Aspergillus fumigatus	80	66.66	40	40	40
Aspergillus ochraceous	61.9	11.11	11.11	11.11	11.11
Penicillium oxalicum	50	21.42	0	0	0
Fusarium semitectum	50	33.33	15.78	15.78	15.78
Curvularia lunata	46.87	22.72	-6.25	-6.25	-6.25
Aspergillus niger	46.42	31.81	31.81	31.81	31.81
Trichoderma sp.	45	15.38	15.38	15.38	15.38
Curvularia eragrostidis	42.85	7.69	4	4	4
Paecilomyces variotii	36.36	17.64	17.64	17.64	17.64
Aspergillus tamarii	22.72	2.13	0	0	0

After fumigation of 48ppm of eucalyptus oil among 100 numbers of fungal spores of ten isolated strains, *Aspergillus fumigates* had again showed the highest PMI, followed by *Aspergillus niger* , *Aspergillus fumigatus* , *Penicillium oxalicum, Trichoderma sp, Fusarium semitectum* , *Paecilomyces variotii* , *Curvularia lunata* , *Aspergillus ochraceous* and *Curvularia eragrostidis* (Table 1). After 4th day the PMI value decreased and the re-growth of mycelia had been observed. The re-growth was not the same for all species. *Aspergillus tamari, Aspergillus niger, Penicillium oxalicum, Trichoderma sp., Fusarium semitectum, Paecilomyces variotii, Aspergillus ochraceous* and *Aspergillus fumigates* had started to grow after the 4th day of fumigation but their growth was slow and shown the same PMI value till the 21st day (Table 2).

Table 2. Fumigation of 48ppm of eucalyptus oil per 100±20 spore of each isolate

Species	2 day	4 day	6 day	14 day	21 day
Aspergillus tamarii	29.16	10.52	5.55	5.55	5.55
Aspergillus niger	34.78	11.76	11.76	11.76	11.76
Curvularia eragrostidis	44.18	25	4	2.04	2.04
Penicillium oxalicum	52.17	26.66	26.66	26.66	26.66
Trichoderma sp.	52.17	26.66	26.66	26.66	26.66
Fusarium semitectum	55.55	40.74	40.74	40.74	40.74
Paecilomyces variotii	57.57	41.66	41.66	41.66	41.66
Curvularia lunata	58.53	34.61	32	32	32
Aspergillus ochraceous	77.14	75	71.42	71.42	71.42
Aspergillus fumigatus	83.33	66.66	40	40	40

All the isolated fungi have shown higher PMI values as the eucalyptus oil dose increased ($R^2 = 0.5$ and $p > 0.5$). After the 4th day of fumigation all the tested fungi have stopped their growth. The same trend had been found in both 72pp and 96ppm per 100±20 spore load (data is not shown).

Several works have demonstrated that eucalyptus essential oil could be used as a potential antimicrobial compound against food spoilage yeasts [16], the white rot fungi in the ware house and other food spoilage microorganism [26]. Very few data has been found regarding the application of eucalyptus oil conserving the museum objects [15, 27]. Similar to our study, *Rogawansamy et al* also examined the effect of the vapour phase of Spearmint, Tea tree, Pine and Cinnamon on the growth of eight common post harvest fungal pathogens growing *in vitro*. They controlled the growth of *Botrytis cinerea*, *Fusarium solani*, *Colletotrichum* sp., *Geotrichum candidum*, *Rhizopus oryzae*, *Aspergillus niger* and *Cladosporium cladosporiodes* more effectively [28].

Principal component analysis for optimizing fungistatic function of Eucalyptus oil. In order to confirm the interactive effects between the three variables (dose of eucalyptus oil, incubation time and PMI) on the growth of ten isolated fungal species a principal component analysis (PCA) was carried out. Figure 4 reports the PCA loading plots on the first three factors of the samples. As expected, factor 1 (eucalyptus oil dose) accounted for the great part of variability (about 59.7%) while factor 2 (time of incubation) had also an effect (25%) on the PMI of the present study. In particular, four clusters have been identified and categorized as very effective, effective, moderate and less effective zone. In the first cluster eucalyptus oil dose and incubation time were grouped for all ten isolated fungi. In the second cluster, PMI or chances of fungal re-growth per day were grouped with the several doses of eucalyptus oil. At last, clusters 3 and 4 were characterized by pronounced differences with respect to the applied dose and incubation time. This analysis ultimately helps to obtain maximum antifungal effect of commercial eucalyptus oil within the minimum interval frequency for the isolated fungi from the strong room of the *Jorasanko* Museum. This statistical analysis (Fig. 4) revealed that 48ppm eucalyptus oil was sufficient to control the fungal load in strong room of the museum. The optimum fumigation frequency was 4 days interval.

Comparative evaluation after fumigation of Thymol and Eucalyptus Oil. Both the applied fungicides, thymol and Eucalyptus oil, removed all the isolated fungi (Fig. 5).

The removal efficiency was not the same. *Fusarium semitectum*, *Aspergillus niger*, *Aspergillus ochraceous*, *Curvularia eragrostidis* and *Trichoderma sp.*were were very poorly removed (<10%) where as other five isolated fungi were removed better after 48 hours of the fumigation of thymol in the strong room (Fig. 3). In laboratory condition *Fusarium semitectum* and *Aspergillus ochraceous* were poorly removed (<10%) by fumigation of eucalyptus oil followed by *Curvularia lunata* (10%), *Aspergillus niger* (12%), *Curvularia eragrostidis* (13%), *Paecilomyces variotii* (14%), both *Aspergillus fumigatus* and *Penicillium oxalicum* (18%). The two isolated fungi *Aspergillus tamari* (32%) and *Trichoderma sp* (64%) were removed well, after 48 hours of fumigation of eucalyptus oil (Fig. 5).

Thymol fumigation is an efficient method to treat different libraries and archives for fungi. Large numbers of items can be treated at one time, in either fumigation chambers or by sealing areas of the building and fumigating entire collections. After fumigation, possible alteration or damage of materials, toxicity to staff and users can be observed. Thymol is irritating to humans when exposed by inhalation, dermal or eye contact. The dermal risk to humans would be Toxicity Category III [29].

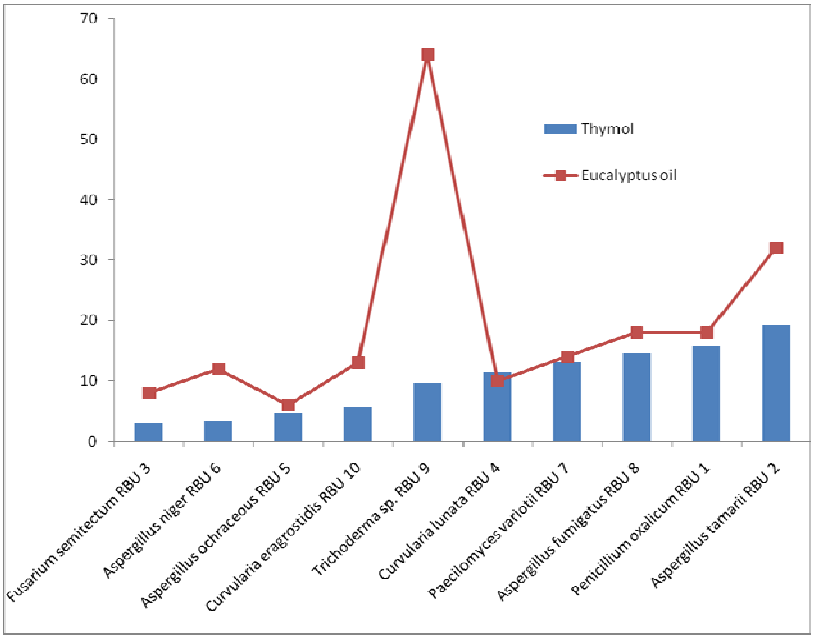

Fig. 5. Comparative study among the removal efficiency (%) after fumigation of Thymol (30ppm) and Eucalyptus Oil (48ppm) on room100±20 spore of each isolate

Beside this thymol also aggravates the foxing of the paper available in library and archives [30-32]. Long term use of antifungal agent creates resistant strains. Zotti *et al* had isolated fungi both from foxed old paper and recently covered cardboard backing in a frame. They identified ten species of filamentous fungi genera formed on the aged paper having resistance to the fungistatic chemicals [33]. Comparatively, eucalyptus oil as a new product has less mammalian toxicity and short environmental persistence and can be a fair alternative for museum also [34].

Conclusions

The results of this work depicted that eucalyptus oil could be used as a potential antifungal compound to control the fungi from strong room of *Jorasanko* museum (both in-vitro and in-vivo). The existing fumigation system is using thymol described as category-III toxic agent, which has been reported as foxing of papery materials by decolorizing them. Long term use of this antifungal agent is harmful for the museum objects. It may also increase the health risk of the museum staffs and partially to the visitors. The proposed eucalyptus oil fumigation method is very much effective as only 48ppm of it has satisfactory fungistatic function. The optimum fumigation frequency was observed at an interval of 4 days. The method is sufficiently sensitive for the individual strain also. The growth of the species like *Aspergillus tamari* and *Trichoderma sp* was reduced up to 32% and 64% which was not even controlled by thymol fumigation. Due to the easily availability and commercial viability, eucalyptus oil has

the better application potentiality with respect to thymol and can be recommended for sustainable conservation of museum objects.

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