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Disinfection of fungal spores on fibrous filters by *Melaleuca alternifolia* mist

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Various infectious microorganisms are transmitted via airborne route and may cause a wide range of illnesses, especially when deposited in the respiratory tract. Commercial HVAC systems could therefore be ones of the entry points for hazardous contaminants such as biological weapon agents (BWA). Cleaning of air by domestic HVAC systems is usually performed on the filters with a range of efficiencies starting from relatively low values. However, when filters are used for collection of biological aerosols, capturing of viable particles on its own is not sufficient for effective control of environment. The reason for it is based on a fact that collected particles could be detached from the filter surface and re-entry the air stream causing contamination of ambient air and corresponding human and animal diseases. This issue call for further investigations related to development of mechanisms of rapid and reliable inactivation of collected particles on the filter surface. Our previous investigations confirmed that bacterial aerosols could be efficiently inactivated if fibres of the filter are coated with *Melaleuca alternifolia*, known as tea tree oil (TTO). The essential oil of, is commonly used as a topical antiseptic, and also as a complementary medicine for bacterial and fungal infections. Several investigations have confirmed the in-vitro activity of TTO against a wide range of common postharvest pathogens. Even a robust strain of *Bacillus subtilis var niger* bacteria was efficiently inactivated during short residence time periods (Pyankov et al., 2008). The current project is focused on the investigation of the possibility of inactivation of robust fungi by TTO coated fibres of common HVAC fibrous filter. Considering the fact that fungal spores are very robust and resistant to chemical and physical stresses, the conclusion about the possibility of their inactivation by TTO could not be simply drawn from the results obtained for bacterial strains.

The experimental program was similar to the one described in detail in Pyankov et al. (2008). In brief, four HVAC filters were installed in parallel in a holder and the TTO was applied onto their surfaces for 5 minutes through the Collison nebulizer. Parallel arrangement of the filters and equal flow rate across all of them ensured uniform distribution of TTO and fungal spores on their surfaces. After 5 minutes run, the nebulizer flow was discontinued and fungal spores, aerosolized from the surface of an agar plate, were mixed with HEPA filtered and dried air and passed through the parallel filter assembly for 3 minutes. On completion of this procedure, filter holder was disconnected and the first filter was immediately placed in a vessel containing 50 ml of sterile water to eliminate any further influence of TTO. The vessel was sonicated in a bath for 2 minutes and then centrifugated for 3minutes. Water and oil were carefully pored from the vessel leaving the entire amount of fungal spores at the bottom. Ten milliliters of fresh distilled water were then added to the vessel and sonicated for five minutes ensuring uniform distribution of the microorganism in it. Then, the liquid was decimally diluted and 100 µl of each dilution was spread on the surface of the agar plate to obtain counts of viable fingi. Three plates were used for each dilution to ensure statistically reliable results. The second filter was undergone though the same procedure 15 minutes after to evaluate the inactivation effect of TTO over this time period. The third and fourth filters were analyzed after 30 and 60 minutes respectively. The final results were then obtained as ratios of 15, 30 and 60 minut results by the result obtained for zero time period (first filter).

For comparison of the results, the experiment was repeated identically for biologically inactive light mineral oil (LMO, Sigma Chemicals M3516).

Obviously, it was impossible to keep the concentration of airborne fungi identical for all experimental runs. To make the concentration related correction, the total concentration was monitored in all experiments by APS (TSI, USA) and viable particle concentration by personal bioaerosol sampler (Agranovski et al., 2005).

The results were obtained for two common environmental fungal strains, i.e. *Aspergillus Niger* and *Rhizopus Stolonifer*. It was found that the TTO is capable to disinfect robust fungal strains collected on the filter surface. The rate of inactivation, however, was not very high and did not exceed 50% for the entire duration of 60 minutes exposure.

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