Pilot study evaluating the efficacy of automated hydrogen peroxide vapor biodecontamination in a cannabis growing facility

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Abstract

State legalization of commercial cannabis production and recreational use of marijuana in areas within the United States is driving expansion and investment in cannabis production facilities. Commercial cannabis crops are susceptible to deterioration and loss due to contamination with microbiological organisms such as molds and fungi, and insects such as mites. Prevention and elimination of these organisms is key to the successful growth of cannabis within a commercial operation. Automated hydrogen peroxide vapor disinfection technology has been in use for over two decades within the pharmaceutical and healthcare industries to control microbiological contamination and produce aseptic operating environments. A pilot study conducted within a commercial cannabis growing facility investigated the efficacy and impact, both on facility infrastructure and crop production, of 35% hydrogen peroxide vapor. Three decontamination cycles were conducted in grow rooms and warehouse space within the facility. Total kill of biological indicators containing $1x10^6$ spores of *Geobacillus stearothermophilus* was achieved in all cycles. No adverse effects were identified related to subsequent crop growth or facility materials. Residue-free, hydrogen peroxide vapor decontamination technology may provide a novel method for controlling and eliminating microbiological contamination of cannabis production facilities.

Introduction

In 2012, the legalization of recreational use of cannabis was introduced in Colorado and Washington state. From 2014 to 2017 a further six states legalized recreational use and many more allow the use of cannabis for medical applications (Drugpolicy, 2018). The legalization of cannabis has driven the expansion of commercial cannabis farming within the United States. With the price of legally produced cannabis around the $1500 to $3000 USD per pound (Cannabis Benchmarks, 2018) both new and traditional farmers are developing growing facilities for this high value crop. Contamination of the crop with microbiological organisms can cause both commercial loss and present infection risks to users of the product, particularly immunocompromised individuals (Cescon, 2008; Sutton, 1986).

Powdery mildews (*Ascomycota*) are a common and important group responsible for cannabis crop infection and loss. The fungus lives on the surface of the plant leaves, reducing photosynthesis and transpiration and removing nutrient, resulting in considerable loss of productivity. The powdery appearance of the fungus is due to the conidia or spore containing conidiophores, which facilitate distribution and reproduction of the fungus. The conidia are primarily distributed via the air and as such can reside within the environment and on surfaces. Kwon-Chung (2013) identifies that conidia from *Aspergillus* species have been shown to survive for 18 years in liquid nitrogen and 60 years post lyophilization. This extreme environmental resistance combined with its ease of airborne spread makes fungal control within growing facilities challenging.

Hydrogen peroxide vapor technology has been in use within the Pharmaceutical and Healthcare Industries for over 20 years. A hydrogen peroxide vaporization module is located within the target enclosure to be decontaminated and produces a vapor from 35% liquid hydrogen peroxide. The sealed enclosure is filled with vapor, whereupon saturated conditions occur and an invisible micro-condensation of hydrogen peroxide lays down onto all surfaces within the enclosure – including any microbiological organisms within the enclosure (Watling, 2002). The hydrogen peroxide remains on the surface for a short period of time, referred to as the dwell period, destroying any contaminating microorganisms. Aeration units convert the hydrogen peroxide into oxygen and water (as humidity) leaving an aseptic enclosure ready for use. A large body of efficacy data exists against a wide range of microbiological organisms, including those of the group Ascomyota, such as *Aspergillus* and *Penicillium* (Bioquell, 2018; Otter, 2009; Hall, 2008; Passeratti, 2013). The efficacy of hydrogen peroxide vapor systems and cycles is validated in the same way as steam sterilizers, using spore based biological indicators. The US FDA requires that sterile drug products are produced in an aseptic processing enclosure validated using at least a 4 $\log^{10}$ (i.e. 10,000 fold) reduction of spores – usually *Geobacillus stearothermophilus* or *Bacillus atrophaeus* (FDA, 2018).

Standard treatments for fungal infections of cannabis crop and / or the growing environment include sulphur burns and wiping down of leaves and surfaces with hydrogen peroxide and neem oil. These procedures can be time consuming and inconsistent, lacking repeatability and validation.

A pilot study was undertaken to determine the feasibility using hydrogen peroxide vapor to control microbiological contamination within a commercial cannabis growing facility.

Materials & Methods

Test facility

The pilot study was conducted in a commercial cannabis growing facility (Oregon Cannabis Authority, Oregon, USA). Three areas were identified for the study, consisting of two grow rooms and one processing warehouse. The building construction was prefabricated steel panelling on a steel frame. Internal walls were plywood, with PVC sheeting cladding the walls to a height of 2.5m and a cement pad floor. The grow rooms were 81 square meters, with a volume of 243m^3 each. The processing warehouse had a volume of 260m^3. The facility was temperature and humidity controlled to 50-65% RH and 21-26˚C via a wall mounted air conditioning system. A HEPA filtered air supply is provided to the grow rooms, maintaining positive pressure relative to the connecting corridors.

Hydrogen peroxide vapor

Bioquell HPV-AQ sterilant (Bioquell Inc, PA, USA), an EPA registered 35% hydrogen peroxide liquid,

was installed into a Bioquell Z-2 hydrogen peroxide vapor generator (Bioquell Inc, PA, USA). The hydrogen peroxide vapor generator possesses on-board catalytic conversion to break down hydrogen peroxide at the end of the cycle.

Biological Indicators

Process efficacy was validated using biological indicators (Bioquell Inc, PA, USA). Each indicator consisted of a 9mm diameter stainless steel disc inoculated with >1 x 10^6 spores of *Geobacillus stearothermophilus* presented in a vapor permeable Tyvek pouch.

Process

On completion of crop harvest, the growing tubs and associated soil were removed from the enclosure (grow rooms). Manual cleaning involving brushing and sweeping was carried out to remove any remaining soil or visible organic material from the area. The hydrogen peroxide vapor generator was placed into the center of the grow room (see figure 1). Twenty biological indicators were located throughout the enclosure including in room corners, on ceiling lights, walls, floor, etc. All personnel vacated the room; the HEPA air filtration and air conditioning units were turned off, and the room entrance was sealed. The hydrogen peroxide vapor generator was activated and injected 10.7g/m^3 into the enclosure, over a contact period of 260 minutes. The catalytic conversion units were activated, converting the hydrogen peroxide into oxygen and water. The room was re-entered when the hydrogen peroxide concentration within the enclosure was <1.0ppm, the legal 8 hour occupational exposure limit for hydrogen peroxide. The biological indicators were carefully recovered and transferred into individual 10ml vials of Tryptone Soya Broth (Biomerieux, Basingstoke, UK) and incubated at 55˚C (±2˚C) for 7 days.

The decontamination experiment was repeated for the second grow room and the processing warehouse. Thirty *Geobacillus stearothermophilus* biological indicators were used in the larger volume processing warehouse.

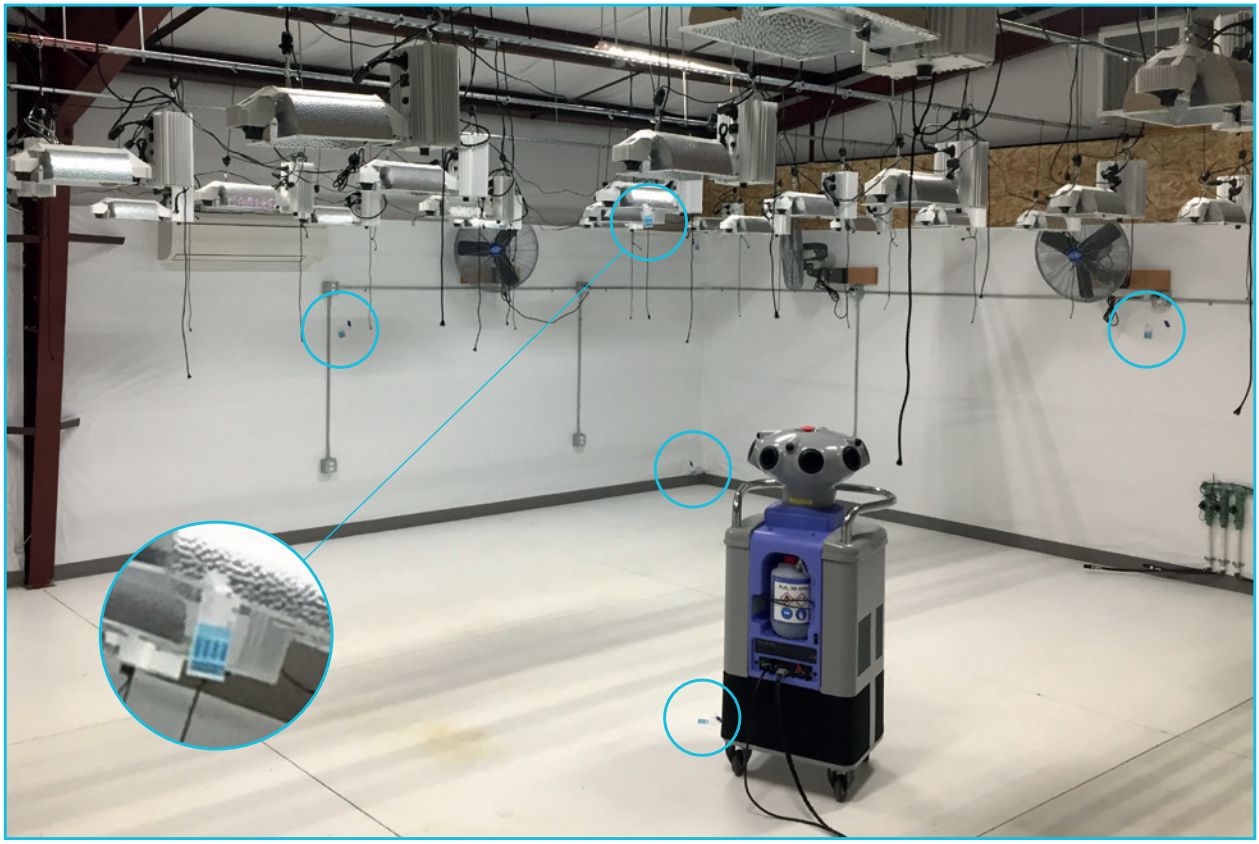

Figure 1. *Bioquell hydrogen peroxide vapor generator located within one of the grow rooms. Biological indicators are positioned at various locations around the enclosure (marked, but not all shown).*

Results

All seventy biological indicators, across the three decontamination experiments, showed full kill after seven days incubation, indicating the complete elimination of spores throughout the enclosures.

Materials within the enclosures showed no adverse degradation or effects from the biodecontamination.

Subsequent cannabis crops showed no adverse effect or variation due to the hydrogen peroxide decontamination cycle.

Discussion

The results of the pilot investigation show that hydrogen peroxide vapor is a viable method for eliminating microbiological contamination such as fungal spores from the manufacturing facilities of commercial cannabis farms. The >1 million spores per biological indicator is a significant challenge and, as previously described, is the measure used to validate the sterilization of medical devices and pharmaceutical manufacturing enclosures.

Hydrogen peroxide vapor is an oxidizer and must contact a microbiological organism for the oxidative based kill to occur. Soil and organic material present within the enclosure during the hydrogen peroxide vapor application process may occlude or hide target organisms preventing contact and kill. Further, organic substances will also be oxidized by the process and thus act as a "sink" for the hydrogen peroxide, depleting its concentration. This can be considered a limitation of the technology - the target enclosure must be physically clean and free of organics – in comparison to sulphur burns, where the plants may remain during the exposure.

The cycle time for the experimental process was approximately 8 hours and thus suitable for an overnight based disinfection schedule. The addition of further aeration units can reduce the overall cycle time. Hydrogen peroxide vapor decontamination is used to reset the bioburden within a facility, such as a hospital or drug compounding pharmacy as a preventative measure and can be utilized within commercial cannabis facilities in the same manner. The hydrogen peroxide vapor process provides an aseptic environment, providing growers with the assurance that their crop will not be contaminated by anything within the growing room. Producers must ensure that they have well controlled entry and exit protocols for the growing areas, as any contamination brought in post the hydrogen peroxide vapor process could potentially affect crop yields.

A concern for the commercial operators involved in the study was material compatibility; and, whether the process would adversely affect the expensive operating equipment within the facilities. No adverse effects were identified with any materials, which is in line with the findings of Yale University Hospital, who have been using hydrogen peroxide vapor to decontaminate their Intensive Care Units for over 10 years (Boyce, 2014). As the hydrogen peroxide vapor is converted into oxygen and water it is residue free, with no chemical substances remaining that could affect the subsequent crops.

Conclusion

There is a substantial body of evidence supporting the microbiological efficacy of 35% hydrogen peroxide vapor and its use within the hospital and pharmaceutical sectors. This pilot study indicates that 35% hydrogen peroxide vapor may be a viable decontamination technology for the control and removal of contaminating microbiological pathogens from cannabis growing facilities.

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Conflict of Interest

C. Harder and R. Harder have no conflict of interest to declare. L. Durr and J. Chewins are employed by Bioquell, a provider of hydrogen peroxide vapor biodecontamination equipment.

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