Can a post-harvest sanitization with Peracetic Acid spray reduce the microbial load in Cannabis sativa

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31st May 2024

Abstract

Cultivation of Cannabis sativa (cannabis) with sufficiently low Total Yeast and Mold Count (TYMC) and Total Aerobic Microbial Count (TAMC) for medicinal use is challenging, even in tightly controlled indoor environments. Stringent European Pharmacopoeia (§5.1.4, §5.1.8) standards as the basis for the New Zealand Minimum Quality Standards (NZMQS) are designed to protect immunocompromised patients and ensure product stability.

Novel pre- and post-harvest treatment methods may help cultivators avoid product irradiation or destruction. While hydrogen peroxide treatments have been used to reduce microbial load, they often lack the efficacy to meet these stringent standards.

1. Introduction

The NZMQS for medicinal cannabis are based on the European Pharmacopoeia (10th Edition), with TAMC limits of NMT 10^2 (200) CFU/g and TYMC limits of NMT 10^1 (20) CFU/g (European Pharmacopoeia §5.1.4, §5.1.8a). Meeting these limits is essential for patient safety and product longevity. Some jurisdictions may apply even more stringent limits.

Cultivating cannabis that consistently meets these standards, even in controlled environments, can be challenging. Peracetic Acid (PAA), a potent oxidizer with known antimicrobial properties, offers a potential treatment solution. This preliminary study investigates the potential of post-harvest Peracetic Acid treatment to achieve a substantial reduction in microbial load in Cannabis sativa, aiming for a 5-6 log reduction. PAA's short withholding period, established safety profile, and existing uses in food sanitation make it a potential decontamination method for cannabis.

1.1. Study Limitations

While this study aimed to evaluate the efficacy of PAA in reducing microbial contamination, it is important to acknowledge several limitations that may affect the generalizability and interpretation of the findings. First, the research was conducted using a single strain of cannabis, and a limited number of biological replicates which may not fully represent the diversity of cannabis cultivars or growing conditions. Second, only one PAA concentration and application method were tested, leaving room for further optimization of application methods, rates and dilutions. Third, the impact of PAA treatment on specific microbial species and on the cannabinoid and terpene profiles of the cannabis flower was not assessed. Additionally, the potential for residual PAA on the treated flower, and the effects of such residues on consumer safety, especially through inhalation or vaping, were not investigated. Finally, while the treatment area was sterilized, environmental conditions within the grow tent were not rigorously monitored, which could potentially introduce variability in the results.

Despite these limitations, this study serves as a crucial initial step in understanding the potential of PAA as a post-harvest decontamination method for cannabis. The results provide a strong foundation for future research to address these limitations and further explore the potential of PAA in ensuring the safety and quality of medicinal cannabis products.

2. Materials and Methods

2.1 Plant Materials

This research was conducted in an approved Cultivation facility, licensed under the New Zealand Medicinal Cannabis Scheme. Dried flower biomass, both trimmed and untrimmed, was used as a starter material for testing (where trimmed flower has been machine trimmed and hand-manicured, while untrimmed flower has larger leaves removed but retains sugar leaves). All materials were initially dried and cured to below 0.65 aW (water activity) to limit additional microbial growth. The trimmed and untrimmed products were placed into separate large bags and left for 72 hours to ensure even moisture distribution. For each trial, the biomass was sorted into 908g (2lb) batches, which represent a suitable quantity for potential large-scale treatment. This process was repeated twice [N=3]. A total of 6356g of biomass was used for sampling; however, the initial biomass bags contained more material. This ensured that when measuring amounts for each test, the biomass did not need to be returned to the initial bags which could impact the materials microbial levels, and could instead be removed from the test.

2.2 Treatment Area Setup

A grow tent (approximately 1m x 1m floor area) was designated as the treatment application area. The tent's interior was sterilized with 0.5% Peracetic Acid (PAA) to eliminate pre-existing microbial contamination. A plastic tray, also treated with PAA, was placed on the tent's base to hold untreated biomass to be sampled from. To ensure clean airflow, a 150mm fan equipped with a HEPA filter (99.97% efficiency at 0.3 microns) was installed to positively pressurize the tent. A multi-tier drying rack, also treated with 0.5% PAA, was placed inside the tent. Each tier was used only once. The tent was left to dry for 24 hours, allowing sufficient PAA evaporation (based on prior observations). The surrounding "lung room" was maintained at 20°C with 60% relative humidity, providing an environment conducive to biomass drying. For precise PAA application, a 300mL Mister 360 spray bottle was used for the capacity to deliver a fine mist of 1mL (v/w) per spray. Nitrile gloves were worn throughout the process.

2.3 Treatment Procedures

The untreated (control) biomass was weighed and placed onto the plastic tray for sampling. All three untreated samples were taken simultaneously. The samples were placed into vacuum-seal bags and heat-sealed without applying a vacuum, replicating standard cultivation facility packaging. Testing was repeated over three consecutive days, with daily testing of both trimmed and untrimmed biomass. Before the following days treatment, all biomass was removed from the tent treatment area and the tiers on the drying rack removed, ensuring only the current samples biomass was exposed.

A 300mL Mister 360 spray bottle was filled with a 0.5% Peracetic Acid (PAA) solution. Trimmed biomass (908g) was placed into one tier, followed by untrimmed biomass (908g) in the tier below. All biomass was laid out flat in a single layer. Each tier received an initial application of 15 sprays (15mL) of PAA. The tiers were then agitated to rotate and redistribute the biomass, followed by a second application of 15 sprays (15mL) per tier. This resulted in a total dosage of 30mL of 0.5% PAA for each 908g batch of dried flower. This initial dosage was chosen as a starting point for further optimization studies.

The treatment tent was sealed, with airflow maintained through the HEPA-filtered intake. The product was left undisturbed for 24 hours to allow sufficient PAA evaporation. A sample of the treated product was tested with a Humimeter RH2 to ensure water activity (aW) remained below 0.65 (with a lower limit of 0.55 aW). This sample was then discarded. Fresh samples from the treated tiers were then vacuum-sealed for testing. The tiers were removed from the treatment tent, and the process was repeated twice more. Surplus biomass was discarded.

2.4 Sampling Procedures

Samples were randomly selected from the tiers, ensuring a representative range of inflorescence sizes. Biomass (20g) was placed into a vacuum-seal bag and heat-sealed, ensuring a small amount of surplus over the receiving laboratory's minimum requirements. Samples were packaged in opaque cardboard boxes to prevent light exposure. Testing was conducted by an independent laboratory certified to both ISO17025 and GMP standards, following European Pharmacopoeia §2.6.31 procedures.

3. Results

The results received (specified as CFU/g, Colony Forming Units per-gram) for testing is as follows, with applicable lab report referenced in brackets by letter:

Table 1: Testing Total Aerobic Microbial Count (TAMC):

Sample	Untreated (Trimmed)	Treated (Untrimmed)	Treated (Trimmed)
Day 1	47,000 {A}	50 {K}	< 50 (LOQ) {D}
Day 2	88,000 {B}	400 {F}	< 50 (LOQ) {L}
Day 3	48,000 {C}	50 {H}	1550 {G}

Table 2: Testing Total Yeasts and Mold Count (TYMC):

Sample	Untreated (Trimmed)	Treated (Untrimmed)	Treated (Trimmed)
Day 1	34,500 {A}	100 {K}	< 50 (LOQ) {D}
Day 2	110,000 {B}	100 {F}	150 {L}
Day 3	31,000 {C}	< 50 (LOQ) {H}	< 50 (LOQ) {G}

Microbial load was reduced in all treated samples compared to untreated controls (Tables 1 and 2). Post-harvest treatment with 0.5% PAA resulted in a dramatic reduction in both Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC) for both trimmed and untrimmed cannabis flower. Many treated samples fell below the limit of quantification (LOQ) of 50 CFU/g.

The following table summarizes the European Pharmacopoeia requirements that dried flower must meet in order to be validated under NZMQS.

Table 3: European Pharmacopoeia limits:

	§5.1.4 - Inhalation	§5.1.8a - Tea (NZ)	§5.1.8c - Tea (Aus)
TAMC limits (CFU/g)	10^2 (200)	10^7 (50,000,000)	10^5 (500,000)
TYMC limits (CFU/g)	10^1 (20)	10^5 (500,000)	10^4 (50,000)

Some variation in treatment results was observed, particularly in the TAMC of Trimmed flower sample from Day 3. This variation could be attributed to several factors, including:

- **Inconsistent PAA application:** The manual spray method may have led to uneven distribution of PAA on the biomass, resulting in some areas receiving a higher or lower dose.
- **Inherent variability:** Natural variation in microbial colonization of the plant material could contribute to differences in treatment efficacy.

4. Conclusion

This study investigated the potential of post-harvest Peracetic Acid (PAA) treatment to reduce microbial load in dried Cannabis sativa. Results (Tables 1 and 2) demonstrate that PAA treatment at 0.5% concentration led to a dramatic 3-5 log reduction in both Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC). This substantial reduction effectively eliminates microbial contamination, even to levels below those mandated by the most stringent standards in the European Pharmacopoeia, suggesting that PAA could be an effective post-harvest sanitization method.

The findings of this study may have significant implications for the cannabis cultivation industry. By demonstrating the efficacy of PAA treatment, this initial research offers cultivators or packing processors a viable and effective method to consistently meet and exceed regulatory requirements for microbial contamination. The short withholding period, established safety profile, and compatibility of PAA with existing food sanitation practices further highlight its potential as a valuable tool for ensuring product safety and quality.

While the majority of samples showed a remarkable reduction in microbial load,

Further research is warranted to optimize both dosage and application methods to minimize this variation. Future investigations could focus on:

- Evaluating alternative application methods, such as ultrasonic misting or heavier droplet sprays, to potentially enhance efficacy and consistency.
- Assessing the potential for automation to streamline the treatment process and ensure uniform application across larger scales.
- Exploring the feasibility of applying PAA treatment immediately pre-harvest as a preventative measure to further reduce initial microbial load.
- Examining the impact of PAA treatment on cannabinoid and terpene profiles to ensure product quality is not compromised.

By addressing these questions, future research can build upon the promising findings of this study and further establish PAA as a valuable tool for ensuring the safety and quality of medicinal cannabis products.

5. Conflicts of interest

Conflict of Interest Statement

The author of this study is involved in the cannabis cultivation industry and may benefit from the wider availability of flower that meets regulatory standards, which could result from the adoption of the PAA treatment method described herein.

The author has no other direct or indirect financial interest to declare.