Aspergillus sp. testing in the emerging _Cannabis sativa_ industry in New York State

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Aspergillus sp. are ubiquitous filamentous fungi found worldwide, and are common in the soil, decaying vegetation, water, and air. The genus *Aspergillus* is comprised of more than 180 different species, but only a few are medically relevant pathogens, such as: *A. fumigatus, A. niger, A. flavus, A. terreus,* and *A. nidulans.* The primary medical concern from an *Aspergillus* sp. infection is Pulmonary Aspergillosis, which results from *Aspergillus* spores infiltrating the Pulmonary alveolar region of the lung [1].

Aspergillus sp. spores are found in indoor and outdoor environments, and in significantly higher concentrations in urban than in rural areas [2], making the aforementioned areas a higher risk for susceptible individuals. *Aspergillus* sp. in indoor environments can be found in the air, on building surfaces and appliances, in drinking water, and dust [2]. The spores are found in purportedly sterile areas such as in hospitals [2] and spacecrafts [3].

Aspergillus sp. is an opportunistic pathogen – i.e., a pathogen that does not cause disease in healthy people but may become problematic in immunocompromised and unhealthy individuals – that mostly affects people suffering from underlying conditions such as cystic fibrosis, tuberculosis, asthma, or chronic obstructive pulmonary disease [4]. Although there were nearly 15,000 hospitalizations due to aspergillosis in the US in 2014, *Aspergillus* sp. is not a threat to most people whose immune system is normally functioning, and who do not suffer from underlying health conditions [4]. Cannabis smokers have been found to develop pulmonary aspergillosis at 3.5 times the usual rate, with these cases also almost exclusively affecting the immunocompromised [5].

Aspergillus sp. can be detected via PCR, or via plating techniques. The PCR techniques are the primary mode of detection because they are highly specific and through the DNA amplification the species can be determined. This allows good assurance that the pathogenic microbe is present. Plating techniques are also commonly used but are traditionally not employed for *Aspergillus* sp. pathogen detection.

Cannabis sativa L. (marijuana, hemp; henceforth cannabis) is currently the largest emerging crop worldwide. This angiosperm (flowering) plant from the dicot family Cannabaceae [6] is one of the first domesticated plants by several ancient cultures [7-9] due to its versatility.

An evidence-based detection approach that include practices, procedures, programs, and policies that have been proven effective at making public health decisions for *Aspergillus* sp. could prove useful in the cannabis industry. Harmonized testing for *Aspergillus* sp. is not the reality across the U.S., with some states requiring species identification while others only require a general screening for Total Yeast and Mold (TYM). While TYM screens can be a good indicator, and *Aspergillus* sp. fall under the TYM category, more specialized testing is typically required to differentiate between pathogenic and non-pathogenic yeasts and molds. Because *Aspergillus* sp.

is ubiquitous, states that require a presence/absence test experience excessive product batch failure, which ultimately hinders the uptake and sustainability of the cannabis industry.

The Office of Cannabis Management (OCM), currently mandates the same testing and acceptance standards for *Aspergillus* sp. in both recreational and medical cannabis products [10]. These requirements mandate no detectable *Aspergillus* sp. through presence/absence via a PCR test [10]. The OCM recognizes that there are many microbes commonly found in the environment that are only opportunistic pathogens for immunocompromised individuals (i.e., the Total Viable Aerobic Bacteria Count, and Total Yeast and Mold Count; Table 1), and so counts for these microbes in recreational cannabis are given for information only, while there is an upper limit set for medical cannabis [10].

Total Aerobic Bacteria and Yeast and Mold Level Numbers in Cannabis Products			
Aerobic Bacteria		**Yeast and Mold**	
Unextracted Average	**Unextracted Median**	**Unextracted Average**	**Unextracted Median**
227,824 CFU/g	110,000 CFU/g	37,922 CFU/g	5,800 CFU/g

Table 1. Average Aerobic Bacteria and Total Yeast and Mold Level Numbers by Biotrax (Buffalo NY; (https://cann-trax.com/) from January 2023-June 2023 on Unextracted (Flower Prerolls, and Kief) and Extracted (Edibles, Topical, Concentrates) products. **Largest Number available is >490,000 cfu/g (Colony Forming Units per gram) and was treated as 490,000 CFU/g.

The data below shows the stark contrast between unextracted and extracted materials in terms of the presence of *Aspergillus* sp. New York state laboratories are responsible for *Aspergillus* sp. testing for which is the subject of most of the failures with over 50% (Table 2).

Aspergillus sp. on Plant Material Positivity Rates						
	Total Tests	*A. niger*	*A. terreus*	*A. flavus*	*A. fumigatus*	*Total Positives*
Total Unextracted PCR Test	296	43	3	12	63	121
2023 Unextracted Positivity Rates	-	14.52%	1.01%	4.05%	21.28%	40.87%
Total Extracted PCR Test	151	0	0	0	0	0
2023 Extracted Positivity Rates	-	0%	0%	0%	0%	0%

Table 2. *Aspergillus sp* numbers by Biotrax (Buffalo NY; (https://cann-trax.com/) from January 2023-June 2023 on Unextracted (Flower Prerolls, and Kief) and Extracted (Edibles, Topical, Concentrates) products.

Remediation

Nearly all extraction methods render an *Aspergillus* sp. test moot. *Aspergillus* sp. is fully destroyed by processes exceeding 130°C [11, 12], and so any material that will see a high temperature distillation can safely be assumed to be *Aspergillus* sp. free. Any combination of a temperature extreme enough to kill the mold (60°C for a prolonged period) and the use of a low micron filter to remove spores should also be sufficient in making an *Aspergillus* sp. free product [11, 12], as well as using a solvent known to kill spores. However, an initial test of ground biomass may still be desirable depending on what additional processes are applied, as the presence of toxins produced by aspergillus may concentrate in non-distilled products.

Remediation are techniques used by many to repair their cannabis products once they have failed for microbial or TYM testing. Gamma irradiation is one of the most used remediation methods. Whether irradiation affects the quality of the product is still debated, with some studies finding that the THC and CBD content is not affected [13], and others finding that the active compounds, including terpenes, were affected [14]. Cannabis can be inhaled and eaten, and remediation for each of these consumption routes may differ given that the respiratory and the gastrointestinal system are very different. Still, remediation including gamma radiation, has been a methodology used as a preventive method in the food industry for tomatoes affected by bacteria [15], strawberries affected by *A. niger* [16] or viruses [17], and raspberries [17, 18], among other foods that are eaten raw and also cultivated outdoors. A big problem with remediation is the exorbitant costs that cultivators must pay to remediate their products, ranging from $125-360/pound, in addition to the multiple other costs faced by cultivators through testing. This is another argument to abolish *Aspergillus* sp. pass/fail testing, and a CFU (Colony Forming Units) threshold should be used for information only.

Potential Approaches

Suggestions to the Cultivators

There are multiple safety measures that cultivators can take to diminish TYM and *Aspergillus* sp. in their products. Pre-harvest precautions if growing indoors include using fans to increase air circulation and the reduction of TYM [19]. Flower products that are hung-dry instead of trimmed while wet have less TYM [19], additionally, trimming while wet damages the inflorescences, resulting in post-harvest microbial buildup [20]. Lastly, the adequate drying process is crucial, assuring that there are no pockets of moisture left in the flower to avoid the survival of TYM [19]. Proper drying conditions include a dry cool environment with appropriate ventilation and ample space between the plants [21].

The handling of the plants during and after harvest should be done with gloves, to avoid transfer of TYM between plants but also the spread of other diseases. Avoiding touching the flowers as much as possible after harvest is recommended, including during drying and storage. Flower should be stored in appropriate containers such as plastic bags that prevent air from getting in, and therefore particles and spores found in the air. These bags could then be placed in plastic totes or barrels for transportation.

Avoiding touching and opening containers holding flower product is also important for retailers. Given that consumers like to smell and see the flowers before purchasing, it is recommended that retailers have two different containers, one to show consumers the flower product, and another with the actual product that would be sold.

Suggestions to the legislators

A Call to Action, for our regulators to put forward evidence-based approaches that consider both protecting the consumer and growing the industry. As previously mentioned, *Aspergillus* sp. are ubiquitous and regulations that test for presence/absence will be counter to a budding marketplace. Possible feasible approaches are suggested below.

Given the lack of data regarding the real-world harm caused by inhalation of *Aspergillus* sp. spores by a healthy population and the harm done to a fledgling market, especially considering the expressed intention of combating a still thriving illicit market [22], the wisest course of action is likely to avoid overuse of precautionary principles and suspend *Aspergillus* sp. pass/fail testing until certain questions have been answered. Below the questions, possible studies and parameters that can be researched to further understand *Aspergillus* sp., its amounts, and risks to Cannabis consumers:

Possible future research studies

-How does remediation affect cannabis flower? Testing multiple strains with diverse chemotypes before and after remediation will allow us to understand whether remediation (and the type of technique used) affects the chemotype and quality of the product.

-How many viable vs. dead spores are found in cannabis flowers? Does spore viability matter? Although inhaling live spores produces a bigger effect than inhaling dead spores [23], dead spores may still pose a threat but its extent for cannabis consumers is still unknown.

-Are the microorganisms and their amounts including yeast, mold, and bacteria in outdoor vs. indoor cultivations different? Given that plants grown in indoor vs. outdoor facilities will face different environments, understanding whether the microorganisms present are different is important for consumers, cultivators, and regulators.

-What type and how abundant are bacteria and yeast, mold -including *Aspergillus* sp.- in extracted products that use different modes of processing? Cannabis processing does alleviate microorganism burden, but the multiple extraction methods have yet to be compared.

-Are long term cannabis consumers, and does consumption mode pose an adverse effect? Because long-time traceability of cannabis consumers has not been done rigorously, the health of these users is unknown, and the mode of consumption in long-term users has not been documented.

-What is the Colony Forming Unit (CFU) count that is harmful for healthy cannabis inhaling consumers? Because, as said, *Aspergillus* sp. are ubiquitous fungi, understanding how many viable spores is OK for consumers to inhale, is crucial.

-Do non-viable *Aspergillus sp.* spores also pose allergic and health threats to healthy consumers? Because even if the *Aspergillus* sp. are dead and non-viable, they will test positive on a PCR presence/absence test but whether they pose a health threat is still unknown.

Until these questions are answered in a compelling way by peer reviewed studies, the following changes are recommended to ensure the health of the New York State cannabis industry. To acknowledge the danger that *Aspergillus* sp. poses when inhaled by immunocompromised individuals, an additional warning label should be applied to all adult-use smokable or otherwise inhalable flower products. The language used should resemble: "Warning: Use under your own discretion; do not consume if immunocompromised or taking immunosuppressants or steroidal treatments." Additional precautions should be taken via general education campaigns alerting consumers to this potential risk, as well as the availability of medical grade marijuana as an alternative for those seeking therapeutic effects. Testing for *Aspergillus* sp. should continue for adult-use products, but this testing should be amended to include a component that ensures the viability of any detected *Aspergillus* sp. The results of these tests should not be determinant of the material's viability for sale but should remain clear and present on the material's CoA to allow consumers to make an informed purchase. Finally, *Aspergillus* sp. testing should no longer be mandated for edible and/or chemically processed cannabis products that have been shown to destroy aspergillus and its spores.

Below the current testing mandates and possible approaches for TYM (Table 3) and bacteria (Table 4)

Cannabis Type	Product	Current Testing Requirement	Recommended Testing Requirements
Medical	Unextracted	10,000 or 10^4	N/A
	Extracted or infused	1,000 or 10^3	N/A
Adult use	Unextracted	Report results	No Change
	Extracted or infused	1,000 or 10^3	No Change

Table 3. New York State Total Yeast and Mold with potential recommendations.

Cannabis Type	Product	Current Testing Requirement	Recommended Testing Requirements
Medical	Unextracted	100,000 or 10^5	N/A
	Extracted or infused	10,000 or 10^4	N/A

Adult use	Unextracted	Report results	No Change
	Extracted or infused	10,000 or 10^4	No Change

Table 4. New York State Total Aerobic Bacteria Levels with potential recommendations.

Therefore, we suggest that the current standards for adult-use flower products of TYM (Table 3) and Bacteria (Table 4) should be for information only. For extracted adult-use products, the maximum CFUs of 10^3 for TYM (Table 3) or 10^4 for Bacteria (Table 4) should remain as-is. As aforementioned, different testing, and perhaps more stringent requirements, may be appropriate for medical products, but such a recommendation is beyond our current scope.

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