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Towards a Cleaner Green: A Review of Microbial Testing of Medical Cannabis and Its Implications for Patient Health

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Abstract: The recent surge in medicinal cannabis uses globally, largely due to changes in supply laws, has necessitated a comprehensive examination of its safety and quality. Despite its legal use in over 40 countries, there is increasing concern about microbiological contaminants, notably fungi, in cannabis. While background (or normal levels) of microbes are considered harmless with proper growing and manufacturing practices, some can pose health risks, particularly in certain applications like smoking, tinctures, or edibles. This literature review aims to explore the occurrence, identification, and health implications of fungal pollutants in medicinal cannabis. It also examines the negative effects of specific mycotoxins and the testing standards for medical cannabis, contributing significantly to current medical practices and future policy development in this rapidly evolving field. The presence of moulds and fungi on cannabis can potentially make users sick, with Botrytis cinerea, Aspergillus, Penicillium, Fusarium and Alternaria being common contaminants. However, the evidence linking exposure to fungus in cannabis with illness after consumption is limited, and the risks seem to depend on individual factors and level of exposure. The medicinal use of cannabis and cannabinoids has been shown to control various biological processes related to cancer and other illnesses. Cannabis is being used as a supplement for chronic pain, anxiety, depression, and other neurological conditions. Nevertheless, solvents, pesticides, and fungal pathogens may contaminate therapeutic cannabis. Mycotoxins from these fungi have been detected in cannabis samples, but their health effects are unknown. In growing medicinal cannabis markets like Thailand, mycotoxins might cause considerable economic losses. To reduce supply chain risks, medical cannabis products require more research on practical microbiological safety recommendations for improved public health.

Keywords: cannabis, fungi, mould, mycotoxins, contamination, public health, medical cannabis, testing

I. INTRODUCTION

In recent years, there has been a substantial increase in the use of cannabis for medicinal purposes worldwide principally due to changes in local supply laws. As a result, there is a growing need for thorough examination of its safety and quality. Currently, the consumption of cannabis for medicinal purposes is legal in over 40 countries and 33 states in the United States, with some industry analysts predicting that the global market will be worth over USD 62.6 billion by 2024 [1]. Researchers are currently exploring the endocannabinoid system in humans, with around one million scientific articles on the subject [2]. However, despite the use of this botanical becoming increasingly prominent in the field of medicine and healthcare, there has been a growing focus on investigating the presence of microbiological contaminants, particularly fungi, and the associated issues that may arise. Although many of these microbiological entities are harmless, provided good growing and manufacturing practices are followed, certain contaminants can pose health risks when included in medical formulations, particularly under specific situations such as for smoking, tinctures or for inclusion in edibles.

The purpose of this literature review is to systematically examine a range of relevant inquiries on the occurrence, identification techniques, and health implications of fungal pollutants in smokeable and medicinal cannabis. This manuscript therefore aims to provide readers with a detailed understanding of the complex relationship between medical cannabis and microbiological safety. It achieves this by conducting a thorough examination of various topics, including the harmful impacts of specific mycotoxins and the accepted testing standards and norms for medical cannabis.

These discoveries play a crucial role in shaping both present medical practices and future policy approaches in this rapidly developing industry.

II. METHODS

The paper will be thematically structured after the Socratic Method where the content is based on a question-and-answer format to key questions. Papers were sourced using PubMed (https://pubmed.ncbi.nlm.nih.gov/), Semantic Scholar (https://www.semanticscholar.org/) and two AI information retrieval tools, Elicit (https://elicit.org/) and Scite (https://scite.ai/). A series of 10 questions were developed and answered based on academic literature that was retrievable using this method. AI-based literature reviews developed in this way were recently discussed in Wagner et al. [3].

III. QUESTIONS

A. What are the top health reasons for use of medical cannabis products?

The top health reasons for medical cannabis use are chronic pain, anxiety, and depression, according to multiple studies [4]-[7]. Reference [4] found that the majority of medical cannabis users in Australia reported using it for chronic pain (55.2%), mental health conditions (35.5%), and sleep problems (27.4%). Similarly, Kosiba et al. [5] conducted a meta-analysis and found that pain (64%), anxiety (50%), and depression/mood (34%) were the most common reasons for medical cannabis use across studies. Reference [6] also found that the top reasons for use among 1429 medical cannabis users in Washington were pain (61.2%), anxiety (58.1%), and depression (50.3%). Ogborne et al. [7] interviewed 50 medical cannabis users and found they used it for conditions like chronic pain, depression, anxiety, insomnia, and everyday stresses.

Beyond pain, anxiety and depression, medical cannabis is used for a variety of other conditions. Lintzeris et al. [4] found it is also used for cancer (3.8%) and nausea/vomiting (12.1%), while other studies show some strains are potentially useful against COVID where they exert anti-inflammatory properties [8]. Reference [9] found it can decrease symptoms like pain, spasticity, and poor appetite in people with Alzheimer's, Parkinson disease, Amyotrophic Lateral Sclerosis, or Multiple sclerosis. Reference [10] states that medical cannabis can treat pain, muscle spasms, nausea, and vomiting related to cancer treatments. Reference [11] found medical cannabis is used for headaches/migraines (35.5%), nausea (27.4%), and muscle spasticity (18.4%) in addition to the top three reasons. Medicinal cannabis also showed potential in the treatment of mental health disorders such as anxiety, depression, and post-traumatic stress disorder (PTSD). Cannabis cannabinoids interact with the endocannabinoid system, which regulates mood and emotions [12].

Medicinal cannabis is also used to treat sleep problems including insomnia [13]. Medicinal cannabis also showed promise in the treatment of neurological illnesses such as epilepsy and multiple sclerosis. CBD, a non-psychoactive cannabinoid, has been shown to be especially beneficial in lowering seizures in epileptic sufferers. Moreover, cannabis-based drugs have been licensed for the treatment of spasticity in Multiple sclerosis patients [14].

In summary, the literature shows the primary health reasons for medical cannabis use are chronic pain, anxiety, and depression. However, it is also used to treat a range of other conditions like cancer, nausea, insomnia, and symptoms related to neurological disorders. Medical cannabis appears to have a wide range of therapeutic benefits according to patient reports and some studies, though more research is still needed.

B. What percentage of medical cannabis is unfit for sale due to contamination? What is the incidence of fungal contamination of medical grade cannabis flower and plant material?

The research on this topic shows that a significant portion of medical cannabis may be contaminated and unfit for sale. McLaren et al. [15] found in a review of international research that while some countries have not monitored cannabis potency or contamination over time, others have found increases in potency and the presence of contaminants. Reference [16] estimated that in California, if a batch of cannabis fails mandatory testing, the cost of destroying the contaminated cannabis accounts for the largest portion of total testing costs.

Several studies analysed samples of confiscated cannabis and found high rates of contamination. Contamination may be from chemicals or microbes, while lab testing may reveal variations in terpene content or THC levels that differ from expected. For example, Mehmedic et al. [17] found in an analysis of over 46,000 cannabis samples from 1993 to 2008 that hashish and hash oil potencies varied considerably, ranging from 2.5% to 29.3% THC. ElSohly et al. [18] found in an analysis of over 38,000 cannabis samples from 1995 to 2014 that while the potency of illicit cannabis plant material has increased over time, the cannabidiol content has decreased, resulting in a change in the THC to CBD ratio from 14 times in 1995 to 80 times in 2014. Pusiak et al. [19] argues that Canada's stringent quality control and assurance measures for cannabis aim to provide consumers with safe products, but notes that as policies progress, more consideration of these measures is needed. In many cases, growers may inadvertently alter the microbiology of the crop through inappropriate selection and use of nutrients or pesticides or chemicals used during processing that may be toxic.

Two studies evaluated cannabis available from regulated medical sources and unregulated recreational sources in the Netherlands. Hazekamp et al. [20] found that cannabis from regulated pharmacies was more reliable and safer, as coffeeshop cannabis was often underweight and contaminated with bacteria and fungi. However, no differences were found in cannabinoid or water content. Reference [20] found that while gamma irradiation of herbal cannabis effectively reduces microbial contamination to meet pharmaceutical standards, it does not alter the content of THC and CBD or the water content and microscopic structure of the flowers. The effects were limited to reducing some terpenes while keeping the terpene profile the same.

Finally, Raber et al. [21] found that cannabis concentrates, which are gaining popularity and often consumed through dabbing, represent a contamination concern. Over 80% of 57 concentrate samples from California dispensaries were contaminated, with THC concentrations ranging from 2.7% to 75.9%. Up to 40% of the theoretically available THC could be inhaled in one dab. While dabbing offers immediate relief to patients, it may also appeal more to recreational users seeking rapid and intense effects. The potential role of microbes being inhaled should not be underestimated.

In summary, research shows high rates of contamination (both chemical and microbiological) in both regulated and unregulated cannabis sources, though regulated medical cannabis may be safer. Stricter quality control measures aim to address this issue but may be insufficient. Cannabis concentrates, represent an area of concern due to their popularity and high risk of contamination. Overall, a significant percentage of medical cannabis appears to be unfit for sale due to the presence of contaminants, though further research is still needed.

C. Has mould or fungus in smokeable cannabis made anyone sick?

There is evidence that moulds and fungi present on cannabis can make people sick. *Botrytis cinerea*, often known as grey mould, is a fungus that may infect cannabis plants, causing substantial quality difficulties as well as post-harvest losses. This fungus has contaminated marijuana buds, rendering them unsafe for ingestion [22]. Moreover, *B. cinerea* aerial conidia may expose cannabis workers to occupational health risks like allergic sensitization and hypersensitivity. This is particularly true in outdoor producing farms, where *B. cinerea* is the most common fungus. McKernan et al. [23] found several species of potentially toxic moulds, including *Aspergillus* and *Penicillium*, present on dispensary-grade cannabis flowers. *Aspergillus* species like *A. fumigatus* can cause chronic pulmonary aspergillosis, a progressive lung disease, in long-term cannabis smokers [24]. McPartland et al. [25] notes that bacteria, moulds and fungi on cannabis may trigger allergic reactions and asthma in some users or even cause opportunistic infections. Kagen et al. [26] found that smoking marijuana contaminated with *Aspergillus* organisms can induce sensitization and a variety of immunologic lung disorders.

However, the risks seem to depend on the individual and level of exposure. While moulds were commonly found on cannabis plants and products, not all users report adverse health effects. Punja et al. [27] found various fungal species present as endophytes within cannabis plants but did not report associated harms. Brochu et al. [28] identified powdery mildew, caused by *Golovinomyces ambrosiae*, on cannabis in Canada, but did not link this to any reported health issues.

There are a few reported cases of cannabis-related diseases that may be linked to mould exposure. Tennstedt et al. [29] notes that long-term cannabis use can cause severe Raynaud's phenomenon and arteritis, though the role of moulds is unclear. Al-Ahmad et al. [30] found that some people developed allergy-like symptoms after exposure to *Stachybotrys chartarum* mould, similar to 'darkroom disease' that causes respiratory issues in those exposed to mouldy, damp environments. However, more evidence is needed to conclusively prove moulds caused these health issues (for cannabis), although the literature on adverse health following exposure to damp and mould affected buildings (poor indoor air quality) is well supported in the literature.

In summary, while potentially harmful moulds and fungi are commonly found contaminating cannabis plants and products, there is limited evidence conclusively proving that they have made users sick. A few case studies suggest mould exposure may trigger respiratory diseases and allergy symptoms in some long-term cannabis users, but more research is needed to confirm a causal link. Mould risks likely depend on the individual, level of exposure, and specific contaminants present. Overall, mould contamination is an area of concern, but has not been shown to broadly or severely impact public health.

D. What mycotoxins have been recovered from medical cannabis flower?

Mycotoxins are toxic compounds produced by fungi that commonly contaminate medicinal plants and herbal medicines. Several studies have detected various mycotoxins in medical cannabis, though at varying levels. Aflatoxins, ochratoxins, and penicillic acid were found in cannabis samples, though often below maximum limits [31]-[33].

Aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus*, were the most frequently detected mycotoxins, found in 11-17% of cannabis samples. Ochratoxin A, from *A. ochraceus* and *Penicillium viridicatum*, contaminated 3-26.7% of samples. No penicillic acid was detected, though its producers *P. viridicatum* and *P. chrysogenum* were present [32].

The predominant fungi contaminating cannabis were *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* species [31]-[33]. *Aspergillus flavus*, *A. parasiticus*, and *A. niger* were most common, found in 70-90% of samples (Punja 2020; Ahmad 2014). Though present, these fungi did not always produce high mycotoxin levels [32]. For comparison, Halt [34] found that in 62 medicinal plant samples and 11 herbal tea samples, toxigenic moulds and mycotoxins were measured. In order of prevalence, *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Absidia*, *Alternaria*, *Cladosporium*, and *Trichoderma* were found. Aflatoxins were found in 11 or 18% of the 62 medicinal plant samples and 1 or 9% of the herbal tea samples from *Aspergillus flavus*. One of the seven, *A. flavus*-contaminated medicinal plant samples tested positive for aflatoxin, ochratoxin, and zearalenone. This research proposed testing medicinal plant material and herbal teas for mould and mycotoxin before use if kept poorly since mould may develop.

Proper storage and handling can prevent mycotoxin contamination [35-36]. Irradiation eliminated fungi in cannabis samples [31]. Ongoing research into companion Chinese herbal medicines not unlike some medical cannabis formulations aims to improve detection and regulation of mycotoxins in such herbal medicines [37].

In summary, aflatoxins and ochratoxins were the primary mycotoxins detected in medical cannabis, though often below hazardous levels. *Aspergillus* and *Penicillium* species were the main contaminating fungi, which may produce mycotoxins under improper conditions. Improved testing and storage methods can curb mycotoxin contamination in cannabis and other herbal medicines.

E. Has anyone Died from Mould on Cannabis?

The research on this topic is mixed, with some studies suggesting cannabis alone rarely directly causes death [38-39], while others indicate cannabis can be an indirect contributing factor in death due to traumatic injury or exacerbation of pre-existing cardiac conditions [40]-[43]. However, several case studies demonstrate that long term cannabis use or exposure to contaminated cannabis can lead to chronic fungal infections, which may become severe or even fatal if left untreated [44]-[46]. In Nourbakhsh et al. [47], three instances of cannabinoid hyperemesis syndrome (CHS), a disorder characterized by cyclic nausea and vomiting in chronic cannabis users, indicating that CHS may cause or contribute to cannabis user mortality.

Zahra et al. [38] found no deaths were solely attributed to cannabis toxicity in Australia from 2000 to 2018. The cause of 559 cannabis-related deaths were accidental injury (29.9%), suicide (25.0%), or polysubstance toxicity with a mean age of 35.8 years and 81.2% male. Rock et al. [39] similarly found risk of death from cannabis toxicity alone to be negligible in analysis of deaths in England from 1998 to 2020. However, cannabis use was a contributing factor in 4% of cases due to physical injury or exacerbation of cardiac conditions.

One case study showed that on the 75th day following a marrow transplant for chronic myelogenous leukemia, a 34-year-old man developed pulmonary aspergillosis. Before admission, the patient extensively used marijuana for weeks. The marijuana cultures showed *Aspergillus fumigatus* with the same shape and growth characteristics as the open lung biopsy samples. Disseminated illness killed the patient despite rigorous antifungal treatment. Doctors should be aware of this possibly fatal marijuana side effect in weakened hosts [48].

In contrast, Drummer et al. [40] argues that while rare, cannabis use should be considered a potential contributory cause of death in cases of cardiovascular events, citing 35 reported cases of cardiovascular emergencies and at least 13 deaths following cannabis use. Bachs et al. [41] presents six case reports of possible acute cardiovascular death where only cannabis was detected in postmortem toxicology. Tormey in several papers [42], [43] argues that to conclusively attribute sudden cardiac death to cannabis, plasma THC levels should be measured, as urine cannabinoids alone are insufficient.

Finally, case studies demonstrate that long term cannabis use or exposure to contaminated cannabis can lead to chronic fungal infections, which may become severe or even fatal if left untreated. Gargani et al. [44] presents two cases of chronic pulmonary aspergillosis associated with long term cannabis smoking. Benedict et al. [49] found persons who used cannabis were 3.5 times more likely to have a fungal infection, and Reference [46] argues clinicians should be aware of adverse effects of cannabis on skin and mucous membranes, including severe Raynaud's phenomenon and arteritis.

In summary, while direct cannabis toxicity is rarely fatal, the drug can be an indirect contributing factor in death due to physical injury, exacerbation of cardiac conditions, or development of chronic fungal infections. Despite this, indirect hazards from cannabis usage include an increased risk of fatal collisions when driving under the influence [50], [51]. Clinicians should be aware of these potential complications from long term cannabis use or exposure to contaminated cannabis.

F. Allergic respiratory symptoms following cannabis inhalation - could it be fungus allergy?

There is evidence that exposure to fungi, especially thermotolerant species that can grow in the human body, may trigger, or exacerbate respiratory allergies and asthma. Several studies found a link between fungal exposure and allergy symptoms. Decuyper et al. [52] found that while 42% of police officers reported symptoms from occupational cannabis exposure, allergy tests were almost all negative, suggesting the symptoms were from non-immune reactions. However, Sack et al. [53] found that employees at an indoor cannabis grow facility had a high rate of respiratory and allergy symptoms, and some showed signs of cannabis sensitization and probable work-related asthma. Other airway obstruction cases after smoking cannabis appear sporadically in the literature, though the causes are often unclear; and where administration of corticosteroids and antihistamines provides relief, this suggests pre-existing conditions are co-factors. While the research is mixed, some studies point to fungi as a potential culprit [49]. For example, the case study by Stone [54] showed that *Rhizopus* fungus was responsible for causing a pulmonary haemorrhage in a 66-year-old medical cannabis smoker who was also a diabetic. Reference [55] found an association between persistent allergic rhinitis and indoor fungal exposure in Venezuela. Similarly, References [56] and [57] reviewed research showing fungi can trigger allergic rhinitis, asthma, and other respiratory issues. Reference [58] presented a case of a patient with selective allergy to certain cannabis strains, suggesting variability in strains and the need to identify specific cannabis allergens. In the mould allergy literature, the majority of patients with fungal allergy display symptoms like rhinitis or asthma, and *Alternaria alternata* is a common sensitizing fungus. In summary, while the research is inconclusive, several studies suggest exposure to fungi, especially thermotolerant species, may be linked to respiratory and allergy symptoms from cannabis inhalation. More research is needed to definitively prove a causal relationship and determine the mechanisms involved [59], [60].

G. What Microbiological Standards or Tests are used to test Medical Cannabis for testing Yeasts and Moulds in Dried Cannabis Flower?

There are several microbiological tests used to analyse medical cannabis for contaminants and ensure product safety and quality. Reference [61] argues that Canada's current regulations requiring tests for total aerobic count, yeast/mould, coliforms, *E. coli*, and *Salmonella* may not be sufficient, as they do not account for the diverse microbes found in cannabis or for viruses like hepatitis A and Reference [62] explains that cannabis testing is unique from food testing, as cannabis contains antimicrobial compounds and is consumed through various methods beyond ingestion. They recommend molecular microbial enumeration tests in addition to colony forming units to properly assess contamination. To determine recent cannabis use and abstinence, urine, blood, and oral fluids are analysed. This is often to comply with workplace rules and for road safety. Reference [63] states that urine analysis can detect use up to 5 days after intake, while blood analysis is best for determining impairment from acute use. Reference [64] adds that urine THC-COOH levels over 75 ng/mL indicate regular use, while levels under 5 ng/mL show occasional use. They note that hair analysis lacks sensitivity for cannabinoids. Reference [65] found that oral fluid testing with the Draeger DrugTest® 5000 cassette accurately detected cannabis use up to 22 hours after smoking, appropriate for workplace or driving under the influence testing. Notably, testing in persons is around presence or absence and usually for punitive reasons. Testing for mould and other microorganisms that could impact on health would be a worthy public health initiative and would reinforce claims that medical cannabis is a safe product for many people. Pesticide residue analysis is also important to meet safety standards, according to Atapattu et al. [66]. They review methods like liquid-liquid extraction, solid-phase extraction, and QuEChERS for sample preparation, and techniques like GC-MS/MS for analysis. Regulatory limits in Canada are 0.02-3.0 µg/g for dried cannabis and 0.01-2.5 µg/g for cannabis oil. Reference [67] proposes quality specifications for medical cannabis inflorescence from the U.S. Pharmacopeia. They recommend categorizing cannabis into THC-dominant, intermediate, and CBD-dominant chemotypes based on secondary metabolite profiles. Identification, cannabinoid quantification, and contaminant testing, including for pesticides, microbes, mycotoxins, and heavy metals, should be done to ensure safety and enable research.

Reference [68] analysed the microbiome of medical cannabis samples from two California dispensaries. They found the fungal genera *Cryptococcus*, *Mucor*, and *Aspergillus*, which contain opportunistic pathogens, as well as limited bacterial amplification due to plant DNA interference. They recommend whole metagenome analysis to better understand the microbiome.

One of the issues with cannabis testing is that there is a clear delineation between testing for pesticides and other chemical pollutants versus microbiological risks. However, industry is meeting these challenges and there are a range of straightforward testing (culture media) solutions for fungi [69].

In summary, a variety of microbiological tests are recommended to assess medical cannabis safety and quality. These include tests for microbes, pesticides, cannabinoids, and contaminants in cannabis and biological matrices like urine, blood, and oral fluid. Robust testing practices are needed to protect public health, enable research, and meet regulatory standards.

H. What countries test for mould Pathogens on Medicinal Cannabis – e.g. Australia, United States, Thailand?

The economic trend forecast for medical cannabis testing is projected to grow by nearly 12% (compound annual growth) to US3.6B by 2030 [70]. Cannabis testing is essential for ensuring the quality and safety of any cannabis product. The cannabis testing market can be segmented based on test type and end user, enabling businesses to better understand their target customer base. Heavy metal testing is critical in assessing the presence of potentially harmful elements in a sample while microbial analysis helps identify bacteria, fungi or virus pathogens that could have a detrimental effect, while potency testing enables cultivators and manufacturers to ensure the desired levels of THC, CBD, and other cannabinoids. The two main end-user categories for testing are cannabis cultivators and drug manufacturers. In many jurisdictions, cannabis cultivators need to test for safety prior to entering the market, while drug manufacturers use cannabis testing for quality control during production, although lab testing is not a universal prerequisite.

Australia is among the nations that test medicinal cannabis for fungal pathogens [71], [72]. The Therapeutic Goods Administration (TGA) mandates that medicinal cannabis products adhere to TGO 100, which includes testing for Total Yeast and Mold Count (TYMC) and absence of specified microorganisms. The United States is another nation that tests medicinal cannabis for fungal pathogens. Some jurisdictions in the United States mandate yeast and mould testing, but Colorado does not monitor the failure rate. Mould pathogens on medical cannabis are tested in the US but environmental health experts have legitimate concerns that safety testing is lagging behind decriminalisation [73]. Chicago researchers discovered TYM levels in cannabis samples ranging from 23,000 to 64,000 CFU/g [74]. Although several US jurisdictions mandate yeast and mould tests, Colorado does not formally monitor the failure rate. These contaminants can develop during growing, storage or processing. In the US, 36 states monitor for 679 cannabis pollutants in medical or recreational cannabis, including 551 pesticides, 74 solvents, 12 inorganics, 21 microorganisms, 5 mycotoxins, and 16 others. Various jurisdictions have four-order-of-magnitude differences in regulated pollutants and action levels. California samples had 2.3% flower failure and 9.2% extract failure. Boscalid and chlorpyrifos were the most frequent insecticides and fungicides found [75]. Various industry groups have produced useful lists of target organisms and linked regulations per jurisdiction [76]. For example, in California, labs test for a wide range of microbes that can potentially cause harm to users. *Salmonella* spp., *Shiga* toxin producing *E. coli*, and various *Aspergillus* spp., specifically *A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus* must all be tested for to certify the safety of inhalable cannabis products before they reach consumers. Any product that does not pass such testing should not make it to market due to the risk posed to users by presence of these microbes.

In Thailand which recently legalized medicinal cannabis [77], The National Science and Technology Development Agency (NSTDA) established the Cannabis Analytical Testing Laboratory that tests cannabis and hemp for medicinal and associated industries to assure product quality and safety. The ISO/IEC17025-2 laboratory is certified worldwide. The services include potency, terpene, and safety testing (heavy metals, pesticides, residual solvents, mycotoxins and microorganism) [78]. The Thailand cannabis market is perhaps the most significant recent 'green rush' which in 2021 was worth USD 80.3 million and is anticipated to rise at a compound annual growth rate of 58.4% from 2022 to 2030. It is expected that for Thailand which is already the third most visited destination for medical tourism, that further legislation and regulatory clarity, in part to combat against oversupply and illegal imports, will define minimum testing requirements. These potential legislative changes aim to ensure that Thai cannabis is fit for medical purposes [79], [80] and does not contribute to adverse effects [81].

I. Cannabis Testing Labs - benefits to the industry?

Cannabis testing labs provide essential services to the legal cannabis industry by ensuring product safety and quality. According to Valdes-Donoso et al. [82], cannabis testing is mandatory in California and all products must be tested for over 100 contaminants before being sold. Reference [82] estimates the cost of compliance with California’s testing regulations to be $136 per pound of cannabis flower. While testing adds to the cost of legal cannabis, it also adds value by validating product safety.

Cannabis testing labs utilize advanced analytical techniques like mass spectrometry to test cannabis products. According to [83], mass spectrometry can be used to test for potency, pesticides, terpenes, heavy metals, and mycotoxins. Mass spectrometry provides an opportunity for analytical chemists to contribute to the cannabis industry. A discussion about available field and portable testing systems versus handheld or fixed benchtop or lab systems is discussed by Oii et al. [84].

There are many cannabis testing labs that provide software and services to the industry. At the 2021 Cannabis Science Conference in Baltimore, there was significant excitement about the growing potential of the cannabis marketplace, often referred to as the “green rush.” A focus of discussion was the cannabis testing environment, with a spotlight on the primary players and emerging technologies. However, progress in cannabis-based medicine is hampered by a few obstacles, such as the absence of certified professionals, inconsistent regulations, and the high cost of testing equipment [2].

Notably, there is a lack of standardization in testing methodologies and regulations between different jurisdictions. According to Jikomes et al. [85], cannabinoid levels reported by different Washington state testing labs vary systematically. Reference [82] notes that Canadian and U.S. state cannabis regulations differ. Goldman et al. argues that the patchwork of cannabis regulations in North America creates challenges for developing compliant testing methods [86].

In summary, cannabis testing labs and their analytical services are crucial for the legal cannabis industry, though more work is needed to standardize regulations and testing practices across different jurisdictions. When regulations are standardized, cannabis testing can help ensure product safety, quality, and transparency for consumers.

J. The future of cannabis testing for microbes?

The papers present concerning findings on the inadequacy of current microbial testing methods for Cannabis. Reference [87] found several pathogenic and toxigenic bacteria and fungi in Cannabis samples using metagenomic analysis, though these microbes did not grow on standard culture-based testing. Reference [61] argues current Canadian regulations are insufficient, as they do not test for many microbes found in Cannabis like *Clostridium botulinum* or viruses like Hepatitis A.

Reference [88] found colony counts differed by 10-fold between three common growth media, indicating a lack of standardization. Whole genome sequencing showed the media also failed to culture most microbes present. DRBC undercounted yeast and mould, while PDA overcounted due to unchecked bacterial growth. These findings demonstrate the "limitations in the culture-based regulations...superimposed from the food industry" onto Cannabis testing [89].

Reference [90] notes Cannabis' antimicrobial compounds and diverse consumption methods complicate testing. Reference [89] found several toxigenic fungi in dispensary Cannabis flowers using sequencing, though culture-based methods missed them. Overall, the papers argue current microbial testing methods cannot ensure safe Cannabis, especially for immunocompromised consumers, without significant improvements to standardize and modernize techniques. Molecular methods may offer solutions to this "long-standing quantification problem" by detecting endophytes and other microbes that evade culture [88].

In summary, these papers present a compelling case that microbial testing regulations for medical cannabis need revision to better fit this unique product and protect public health. Advancements in sequencing and molecular techniques along with low-cost and portable testing tools seem promising to help address these critical issues.

IV. CONCLUSIONS

Cannabis and cannabinoids have been found to regulate various biological processes such as cell proliferation, migration, invasion, angiogenesis, programmed cell death, and metastatic signalling pathways in cancer preclinical research. As the legalization of medical cannabis expands to more countries, there is a growing interest among cancer patients and their families in using herbal therapy as a supplementary or alternative medication [91]. Numerous studies have demonstrated that chronic pain, anxiety and depression are the primary reasons for using medicinal cannabis, but it is also being increasingly considered for therapy in a range of other mental health disorders ranging from alleviation from social anxiety through to attention deficit hyperactivity disorder (ADHD) [92]. Additionally, it has been used to reduce symptoms of cancer and oncology treatments [93], as well as for nausea, sleeplessness, and various neurological diseases.

However, there is evidence suggesting that a significant portion of medicinal cannabis may be contaminated and unsuitable for sale. Although solvents, pesticides and insecticides are common contaminants, the microbiology of cannabis is less of a known public health threat. While mould and fungus have been found on cannabis, there is currently little proof that they have caused widespread illness. *Aspergillus*, *Penicillium*, *Fusarium*, *Byssochlamys*, or *Alternaria*-type fungi are most cited in the literature. Mycotoxins produced by these fungi, include aflatoxins and ochratoxins, and have been detected in medicinal cannabis samples, although rarely exceeding regulatory thresholds.

However, according to the World Health Organization, 25% of the world's agricultural products are contaminated by mycotoxins [94] and once mould has grown, the mycotoxins cannot be removed, and this problem is the subject of intense research around decontamination [95]. For emerging medical cannabis markets such as Thailand, the threat of mycotoxins cannot be underestimated. For example, regional crop losses from mycotoxins may well exceed 25% as discussed in Reference [96]. In conclusion, further research is necessary to gain a comprehensive understanding of the safety and effectiveness of medicinal cannabis products and what safety guidelines or standards can be practically applied to minimize risk. To this end, further research is needed into cannabis microbiology at the growth, harvest, packaging, distribution, and retail sale phases of the supply chain and to inform policy that promotes public health.

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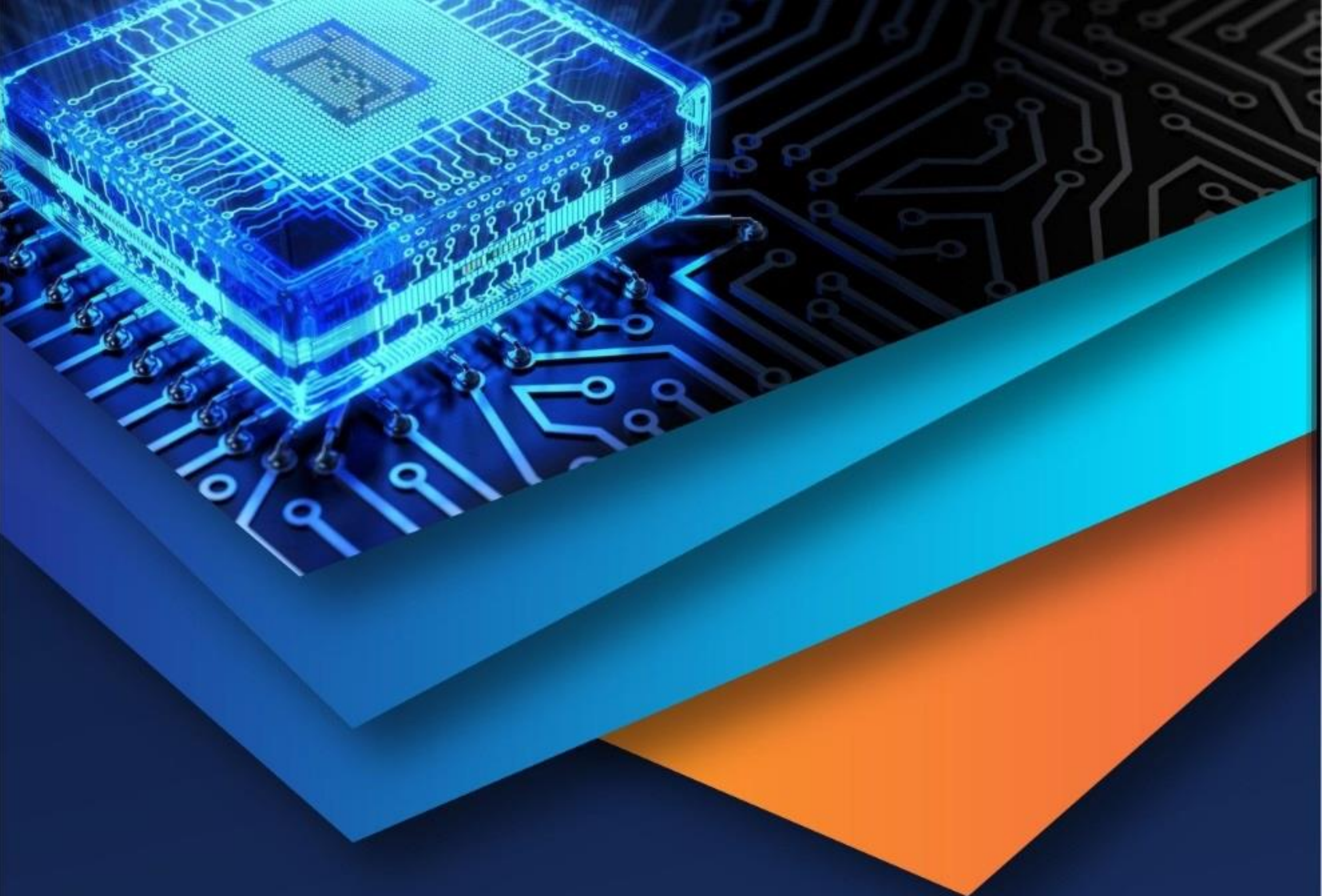

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