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Fungal Infection Diseases- Nightmare for Cannabis Industries: Artificial Intelligence Applications

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Abstract: This review paper highlights the fungal diseases of both indoor and outdoor Cannabis cultivation environments and discusses the Artificial intelligence (AI) based crop disease detection and management. Pathogens are a pain in the neck of every Cannabis breeder. They affect the quality and quantity of yield, thus defeating the aim of cultivation. Some of the fungal pathogen that can attack Cannabis crops are *Botrytis*, *Alternaria*, *Fusarium*, *Penicillium*, *Cladosporium*, and *Aspergillus.* Fungal diseases are Powdery Mildew, Damping off, and Mildew. Of these fungal pathogens, the most common inflorescence disease is gray mold, caused by *Botrytis cinerea. Botrytis cinerea* and *Erysiphe* species complex are currently the most widespread pathogens of Cannabis worldwide. The greatest challenge facing Cannabis and hemp producers is the management of insect pests and pathogens that attack the roots, leaves and inflorescences. The common disease management strategies are-remove and destroy infected plants. Irradiate dried buds with gamma or electro-beam radiation. Another method is to apply biological control agents at rooting and vegetative stages of growth. Pesticides have been found in all Cannabis products, from flowers to edibles, vapes, and smokes. The pesticide pandemic in the Cannabis industry needs urgent attention. Cannabis can contain fungal pathogens and residues of pesticides, fungicides that cause serious and often fatal infections in persons with immunocompromised conditions, such as cancer, transplant, or infection with HIV. Contamination of Cannabis plants and products (i.e., recreational- and pharmaceutical-grades) with mycotoxigenic organisms, including species of *Aspergillus*, *Penicillium*, and *Fusarium*, pose serious health challenges. The manual Cannabis disease identification process is time-consuming and tedious work. Instead, automated methods save both time and effort. The technology of Artificial Intelligence (AI) in the detection and management of disease has already been employed in many crops. The machine learning (ML)-based models were proposed for the identification and classification of plant diseases. The **PlantVillage** dataset is the largest and most studied plant disease dataset, which is used as a reference for the disease detection and management of plant diseases.

Key Words: Artificial intelligence (AI), *Botrytis cinerea*, Cannabis, disease management, fungal pathogens

Introduction

Cannabis sativa L. belongs to family *Cannabaceae* is an annual and predominantly dioecious species originating from **Indian Himalayan Region** and other parts of Asia, China, Tibet, Nepal, Bhutan, Pakistan, Afghanistan, Iran and Persians (1-30, 121-122). *Cannabis sativa* L. has a long domestication history because of its many traditional uses, including textile fibre source, seeds for nutrition, and medicinal or recreational drug use (1-25, 121-122). In recent years, the medicinal applications of *Cannabis sativa* L. have gained wider attention worldwide (1-40). *Cannabis sativa* L., is classified into two types as Industrial

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

Cannabis sativa L. (hemp or fiber type) and, Medical *Cannabis sativa* L. (drug or marijuana) based on its Λ9-tetrahydrocannabinol (Λ9-THC) content (1-48). Medical *Cannabis sativa* L. (drug or marijuana) contains very high levels of Λ9-tetrahydrocannabinol (Λ9-THC) (above 0.3 to 38% of dry weight) (1-20, 121-122). On the other hand, Industrial *Cannabis sativa* L. (Hemp) contains very low levels of Λ9-tetrahydrocannabinol (Λ9-THC) (0 to 0.3% of dry weight) (1-35). However, due to the presence of psychoactive molecules, Λ9-tetrahydrocannabinol (Λ9-THC) and Λ8-tetrahydrocannabinol (Λ8-THC), Cannabis cultivation and its use is restricted/regulated in many countries (1-48, 121-122). Medical *Cannabis sativa* L (marijuana or drug type) is a source of bioactive phytochemicals with promising pharmacological and therapeutic applications (1-25, 121-122). Medical *Cannabis sativa* L (Marijuana or drug type) grown for its medicinal and psychotropic properties of Λ9-tetrahydrocannabinol (Λ9-THC) which are attributed to Cannabinoid and terpene compounds produced in the female inflorescences (1-48). On the other hand, Industrial *Cannabis sativa* (Hemp of fiber type) is grown as a source of fibre and oilseed present in stems and flowers which is considered as the functional food (1-20). Furthermore, hops (*Humulus lupulus*) also belongs to family *Cannabaceae* cultivated for the female cones that produce aromatic oils and alpha acids used in the brewing industry (19-35).

Legalized use of Cannabis products and the rising interest in their therapeutic benefits have opened up new opportunities for therapy and marketing (1-48, 121-122). The changing legal landscape and rising interest in its potential therapeutic utilities have opened new opportunities for therapy (1-50). Multiple Cannabis products are increasingly used in countries that have legalized their use (1-48). This increased production has seen a rise in the incidence and severity of plant pathogens, causing a range of previously unreported diseases (19-48, 53-73). The use and research on Medical *Cannabis sativa* L. (drug or marijuana) is becoming more common, yet there are still many challenges regarding plant diseases of this crop (1-48). Most of the Cannabis that is used recreationally and medicinally is grown at high density in greenhouses or warehouse settings (19-48, 53-72). The cultivation of Cannabis in such a manner resulted in high incidences of infection with fungi, bacteria, viruses, and nematodes (14, 19-48, 53-73). Cannabis particularly Medical *Cannabis sativa* L. (drug or marijuana) can be grown indoors in controlled environment rooms, in greenhouses, as well as hemp in outdoors under natural field conditions (1-48). Hydroponic cultivation is the most commonly used indoors to provide controlled delivery of water, fertilizers, and other chemicals, and substrates such as rockwool, cocofibre blocks, and peat are used as growing media (19-48, 53-73).

To date, there are over **88** known fungal species affecting Medical *Cannabis sativa* L. (drug or marijuana) and hemp plants at all the growth stages, not including storage pathogens (19- 48, 53-73). Of these fungal pathogens, the most common inflorescence disease is gray mold, caused by ***Botrytis cinerea***, which thrives under high humidity and cool to moderate temperatures, and peaks in maritime conditions (19-48, 53-73). On the basis of literature survey, some of the fungal pathogen that can attack Cannabis crops are *Botrytis*, *Alternaria*, *Fusarium*, *Penicillium*, *Cladosporium*, and *Aspergillus*. Fungal diseases are Powdery Mildew, Damping off, and Mildew (19-48, 53-73). Therefore, fungi are nightmare for Cannabis industries and determined to ruin the whole crop. In the following section, the major challenges of microbial load, affecting the health, and major fungal pathogens of Cannabis crop and applications of Artificial intelligence (AI) in the crop disease detection and management have been discussed and updated.

Applications of Artificial Intelligence (AI)

Plant diseases are responsible for 20 to 40% of crop losses globally each year. Disease detection and identification play an essential role in disease management for minimizing crop losses, and since visual inspection is crucial for disease detection. Deep learning (DL) is a natural fit for this problem (17, 18, 120). Artificial intelligence (AI) is a leading technology of the current age of the Fourth Industrial Revolution (Industry 4.0 or 4IR), with the capability of incorporating human behaviour and intelligence into machines or systems (17, 18). The artificial intelligence (AI) revolution started in the early 2010s when convolutional neural networks dominated the computer vision competitions (17, 18,120). The real value of artificial intelligence (AI), however, was understood when it started tackling challenges in other domains such as medicine and physics (17, 18). Today, machine learning (ML) has become an indispensable tool in plant science. It has found wide-ranging applications such as classifying plant cell organelles, high-throughput root phenotyping, and estimating crop growth using drone images (17, 18, 120). Even though machine learning (ML) was used for plant disease identification as early as 2007, and a lack of large public datasets prevented further studies. This changed when the first extensive and public plant disease dataset, **PlantVillage,** was published in 2015 by **Hughes and Salathe** (120).

The **PlantVillage dataset** is the largest and most studied plant disease dataset (120). It contains more than 54,000 images of leaves on a homogenous background (120). There are 38 classes corresponding to plant-disease pairs (120). This sparked a plethora of studies on plant disease classification using deep learning (DL) (17, 18, 120). The rise of deep learning (DL), a subfield of machine learning (ML) and artificial intelligence (AI), is a giant leap towards revolutionizing automation in precision crop disease detection (17, 18, 120). Most of the papers reported classification accuracies above 98% (120).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

Fungal Pathogens of Cannabis

According to the literature survey and study conducted by Punja (2021) (19-48), the most important fungal pathogens currently affecting Cannabis indoor cultivation and production in Canada and other countries are as follows (19-48, 53-73). The predominant major foliage pathogens were identified as *Alternaria alternata* and *Botrytis cinerea*, while the common stem and soil borne pathogens were identified as *Fusarium oxysporum* and *Fusarium solani* (19-48, 53-73). Other important fungi that were isolated from foliage were those producing various mycotoxins that can directly harm patients, such as *Aspergillus* spp. and *Penicillium* spp (19- 48, 53-73).

1) **Damping-off disease**-This disease is caused by Botrytis cinerea, Fusarium oxysporum, Fusarium proliferatum and Fusarium solani.
2) **Powdery- mildew disease:** This is a serious issue and caused by Golovinomyces spp.
3) **Bud rots disease**: Botrytis cinerea, and Fusarium spp.
4) **Fusarium root and Crown rot**: Fusarium oxysporum, Fusarium proliferatum, and Fusarium solani.
5) **Pythium root and Crown rot**: Pythium myriotylum, Pythium dissotocum, and Pythium aphanidermatum.
6) **Post-harvest molds**: Botrytis cinerea, and Penicillium species.
7) **Dudding**: Hop latent viroid.

According to the literature survey and study conducted by Punja and Scott (2023), the root-infecting pathogens included *Fusarium oxysporum*, *Fusarium solani*, *Fusarium brachygibbosum*, *Pythium dissotocum*, *Pythium myriotylum*, and *Pythium aphanidermatum*, which caused root browning, discoloration of the crown and pith tissues, stunting and yellowing of plants, and in some instances, plant death has been reported (14, 19-48, 53-73). On the foliage, powdery mildew, caused by *Golovinomyces cichoracearum*, was the major pathogen observed (19-48, 53-73). On inflorescences, *Penicillium* bud rot (caused by *Penicillium olsonii* and *Penicillium copticola*), *Botrytis* bud rot (*Botrytis cinerea*), and *Fusarium* bud rot (*F. solani*, *F. oxysporum*) were reported to varying extents (19-48, 53-73). Endophytic fungi present in crown, stem, and petiole tissues included soil-colonizing and cellulolytic fungi, such as species of *Chaetomium*, *Trametes*, *Trichoderma*, *Penicillium*, and *Fusarium* (19-48, 53-73). Analysis of air samples in indoor growing environments revealed that species of *Penicillium*, *Cladosporium*, *Aspergillus*, *Fusarium*, *Beauveria*, and *Trichoderma* were present (19-48, 53-73). Fungal communities present in unpasteurized coconut (coco) fiber growing medium are potential sources of mold contamination on Cannabis plants (19-48, 53-73). According to the study conducted by Punja and Scott (2023), swabs taken from greenhouse-grown and indoor buds pre- and post-harvest revealed the presence of *Cladosporium* and up to five species of *Penicillium*, as well as low levels of *Alternaria* species (19-48, 53-73). Mechanical trimming of buds caused an increase in the frequency of *Penicillium* species, presumably by providing entry points through wounds or spreading endophytes from pith tissues (19-48, 53-73). Aerial distribution of pathogen inoculum and mold spores and dissemination through vegetative propagation are important methods of spread, and entry through wound sites on roots, stems, and bud tissues facilitated the pathogen establishment on Cannabis plants (19-48, 53-73).

According to the literature survey, the specific pathogens, such as *Cercopsora cannabina*, have also been reported in agricultural regions worldwide, including Eurasia (e.g., Cambodia, China, India, Pakistan, and Russia), Africa (e.g., Uganda), and North America (e.g., Mississippi and Wisconsin) (19-48, 70-72). Moreover, occurrence of fungal pathobiota, such as Fusaria on *Cannabis sativa* L. host, showed certain site-specificity, as noted in the United States: California—*F. brachygibbosum*, *F. equiseti*, *F. radicicola*, *F. oxysporum*, and *F. solani*; Illinois—*F. oxysporum* f. sp., *Cannabis* F. sp., *F. solani*; and Virginia—*F. sulphureum* (19-48, 70-73). In addition, similar distribution patterns linked with this plant's production system have been detected on a worldwide scale: For example, *Achlya aquatica* as a water mold—India; *Phymatotrichum* sp. causing root rot—Mexico; Pythium spp. inducing damping off—Canada and United States; *Pleosphaerulina cannabina* causing pepper spot—USSR/Russia; *Pseudoperonospora cannabina* responsible for downy mildew—Poland; *Puccinia cynodontis* causing rust and *Verticillium albo-atrum* wilt—China*; Sclerotium bataticola* responsible for charcoal rot—Bulgaria; and *Ramularia collocygni* causing leaf spot—Europe (19-48, 70-72). These examples highlight the importance of preventing spread of unwanted fungal species in emerging Cannabis growing regions (19-48, 53-73).

According to the **Elevated Botanists pest control Handbook**, the other common pathogens and disease of Cannabis crop are Broad mites, Thrips, fungus Gnats, bud rot/ grey mold, Caterpillers and loopers, damping off disease, foliar aphids, Fusarium wilts, hemp russet mites, hemp latent viriod, powdery mildew disease, root aphids, root rot, twospotted spider mites, and whiteflies (**Elevated Botanists**).

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Major Challenge of Fungal Infections of Cannabis

As the demand for Medical *Cannabis sativa* L. (drug or marijuana) increases, many more farms have been established, and with the growing cultivation intensity, challenges and problems have arisen (19-48, 53-73). These challenges are varied and can be elaborated as; 1) Medical *Cannabis sativa* L. (drug or marijuana) is designed for medical purposes, there are strict regulations regarding the growth, quality, and general standards pertaining to the final product, such as pesticide residues, limits on total yeast and mold (TYM) colony forming units (CFUs), and mycotoxin levels present in Medical *Cannabis sativa* (drug or marijuana) dried inflorescences (19-48, 53-73). 2) A lack of theoretical knowledge of Medical *Cannabis sativa* L.(drug or marijuana) plant pathogens and disease reduction methods exists, as there is a lack of scientific research on this subject, and most of the information available refers to the fibre type plants (hemp) grown outdoors, since drug type plants were illegal in the past. 3) The years of Medical *Cannabis sativa* L. (drug or marijuana) inter-crossbreeding and the use of cuttings in commercial farms have led to low plant genetic diversity and increased susceptibility to plant pathogens and pests (19-48, 53-73). Another problem is specifically, it has been reported that Medical *Cannabis sativa* L. (drug or marijuana) plants tend to be less resistant when grown in high concentrations compared to fibre type plants (19-48, 53-73).

Cannabis plants are propagated from cuttings that are rooted and grown vegetatively, following which they are transferred to conditions of specific reduced lighting regimes (photoperiod) to induce flowering (19-48, 53-73). Flower buds are harvested, dried, and stored in vacuum-sealed bags or sealed plastic or glass containers prior to distribution (19-48, 53-73). Fungal infection of roots can occur at any time during the production cycle, while colonization of flower buds generally occurs during the later stages of flower development and can be manifested as a pre-harvest or post-harvest bud rot. In addition, foliar pathogens may infect the plant at any stage during its production (19-48, 53-73).

The greatest challenge remains in reducing microbial loads (colony-forming units) on harvested inflorescences (buds). Contaminating microbes may be introduced during the cultivation and postharvest phases, or constitute resident endophytes (19-48, 53-73). Failure to achieve a minimum threshold of microbes deemed to be safe for the utilization of Cannabis products can arise from conventional and organic cultivation methods, or following applications of beneficial bio-control agents (19-48, 53-73). The current regulatory process for approval of Cannabis products presented a challenge to producers utilizing biological control agents for disease management (19-48, 53-73). The implementation of certified pathogen-free planting material is an important first step, followed by the utility of biological control agents, which still required research to determine their comparative efficacies and modes of action (19-48, 53-73). While postharvest irradiation effectively minimizes mold contaminants, it increases the cost of production and any possible effects on the organoleptic properties of the product need to be assessed. Other treatment options need to be explored for organic producers (19-48, 53-73).

From an economic standpoint, *Botrytis cinerea* and *Erysiphe* species complex are currently the most widespread pathogens of Cannabis worldwide (19-48, 53-73). Damaging flowering buds and stalks, *Botrytis cinerea* causes gray mold and attacks flowers, fresh fruits, and vegetables in hundreds of other hosts, including vineyards, worldwide (19-48, 53-73). *Botrytis cinerea* produces two major phytotoxins: the sesquiterpene **botrydial** and the polyketide **botcinic acid**, which are important virulence factors in *Botrytis cinerea* (19-48, 53-73). The **BcAtf1 gene** has been reported as the global regulator of virulence, controlling various differentiation processes and phytotoxin production in *Botrytis cinerea* (19-48, 53-73). Still, no effective management measure(s) exists against *Botrytis cinerea* or other Cannabis-associated molds due to their genomic plasticity and development of drug resistance (19-48, 53-73). There are no Cannabis varieties resistant to powdery mildew. However, various plant immune pathways can limit the extent of fungal invasion (19- 48, 53-73).

Cannabis Indoor Cultivation: Fungal Pathogens

Cannabis cultivation particularly hemp has started outdoor in the large Agricultural farms (19-48). This is followed by indoor cultivation of Medical *Cannabis sativa* (drug or marijuana) in growing facilities with controlled environmental conditions and supplemental lighting to optimize plant growth (19-48, 53-73). Most indoor cultivation of Medical *Cannabis sativa* L. (drug or marijuana) currently utilizes hydroponic soil-free culture, e.g. rockwool or cocofibre, although soil culture is common, especially for organic production (19-48, 53-73). A combination of indoor environments, which includes expansive greenhouse production, and outdoor (field) environments is used to cultivate Cannabis in Canada, USA, Columbia, China, and Europe (19-48, 53-73). Each production environment faces challenges from plant pathogens, with indoor and greenhouse systems sharing more diseases in common compared to field-grown Cannabis (19- 48, 53-73). In India, organic farming of hemp has been developed and hemp is successfully cultivated in a large agriculture farms (1-18). Cultivation of hemp occurs mostly outdoors, with plants initiated directly from seed or occasionally from transplanting of rooted cuttings produced in greenhouses (1-19,19-48). The major challenges facing expanding cultivation of hemp are seed quality and pressure from weeds, insects and diseases (19- 48, 53-73). Medical *Cannabis sativa* L. (drug or marijuana) as well as recreational Cannabis grown in indoor facilities is exposed to a

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plethora of microbial contaminants occurring on pre- and post-harvest Cannabis inflorescence buds (19-48, 53-73). Furthermore, while desiccation of the flowers and high temperature would be expected to decrease viable microbial counts, it would not eliminate all of the microorganisms nor the endotoxins and mycotoxins that they would produce (or have already produced) (19-48, 53-73). Gamma irradiation, where material is exposed to a high powered gamma radiation source, is another approved method by Health Canada (https://www.canada.ca/) for decontamination which does not cause changes in the content of THC and CBD but does alter terpene quality slightly (19-48, 53-73). As with high temperature drying, this treatment would reduce viable microbial counts, but would not eliminate the dead remains of the microbes nor any endotoxins and mycotoxins that were already present (19-48, 53-73). Other methods, such as cold plasma sterilization, attempt to attain an optimal balance between product activity and safety (19-48, 53-73).

Cultivation of Cannabis indoors begins with vegetative propagation from shoots (cuttings) taken from stock (mother) plants of a desired genotype (strain or chemovar) (19-48, 53-73). Since Cannabis is dioecious in its reproductive mechanism, only female plants that bear unfertilized inflorescences are utilized in commercial production (19-48, 53-73). Male plants are of value solely for selective breeding (19-48). According to the study reported by Punja, (2021) (19-48), these vegetatively propagated strains can harbour undetected or latent fungal or viral pathogens, e.g. *Fusarium oxysporum* causing root and crown rot and Hop latent viroid causing malformation of buds (19-48, 53-73). In indoor growing environments, the introduction of diseased plant materials as cuttings or stock plants can result in the spread of pathogens such as powdery mildew, *Fusarium* spp., Hop latent viroid and potentially other pathogens (19-48, 53-73). Since Cannabis is a short-day (long-night) plant, the 12:12 h photoperiod triggers the onset of inflorescence development, which progresses over an 8-week period to harvest (19-48, 53-73). At maturity, female inflorescences are hand-harvested and dried by hanging them upside down (19-48, 53-73). The buds are then dried to approximately 10% moisture (by weight) in specifically designed drying rooms at ambient humidity of 50–55% and temperatures of 17–24 °C over 5 days (19-48, 53-73). Fungal infection particularly mold is a major problem in the Cannabis product which is a major challenge for the commercial production of Cannabis producers (19- 48, 53-73).

In recent research examining fungal pathogens isolated from Medical *Cannabis sativa* L. (drug or marijuana) inflorescences in Canada, six main pathogens were recovered: *Botrytis cinerea, Fusarium solani, Fusarium oxysporum, Fusarium equiseti, Penicillium copticola,* and *P. olsonii* (19- 48, 53-73). In a similar study, examining potential soil borne and crown rot Cannabis pathogens, four different fungi were reported to cause disease symptoms in Medical *Cannabis sativa* L.(drug or marijuana) plants via root inoculations: *Fusarium* oxysporum, *Fusarium* brachygibbosum, *Fusarium* solani, and *Pythium aphanidermatum* (19 -48, 53-73). Nevertheless, there is a lack of scientific research and knowledge regarding Medical *Cannabis sativa* L. (drug or marijuana) plant diseases, especially in areas where different climatic and ecological niches exist (19-48, 53-73).

Most of the pathogens are fungi and oomycetes, followed by viruses or viroids. Bacterial pathogens are less commonly reported (19-48, 53-73). The most destructive root pathogens are *Fusarium* and *Pythium* species particularly when infections occur during the rooting phase or vegetative growth (19-48, 53-73). The inflorescence infecting pathogens are the most damaging to Cannabis crop as they directly infect and destroy the buds (19-48, 53-73). The most damaging fungi are *Botryis* and *Fusarium* species, as well as a number of other fungi that colonize foliar and flower tissues, including *Penicillium* and *Golovinomyces* species (19-48, 53-73). These fungi produce large number of spores to ensure spread (19-48, 53-73). The extensive development of fungi such as *Fusarium* and *Penicillium* within the inflorescences can also lead to mycotoxin accumulation in the tissues, potentially posing additional health concerns for consumers (14, 19-48, 53-73).

Endophytic Fungal Pathogens of Cannabis

Most of the plant species harbour a suite of microbes (fungal, yeast, bacterial and actinomycete species) present internally that survived tissue surface-sterilization methods used to eliminate external contaminants (19-48, 53-73). These endophytes have generated considerable interest in basic and applied research studies to elucidate their roles within the plant (19-48, 53-73). Microbial residents within the plant can have multiple roles, depending on environmental conditions, growing conditions and host genotype (19- 48, 53-73). For example, *Aspergillus* and *Penicillium* species are amongst the endophytes previously described from Cannabis plants (19-48, 53-73). The tissues harbouring endophytes in Cannabis plants include the pith and surrounding parenchyma cells, which can support the growth of *Penicillium* species in large numbers (19- 48, 53-73). Fungi recovered from pith tissues include species of *Chaetomium*, *Trichoderma*, *Cladosporium* and others (19-48, 53-73). In addition, leaves, stems, petioles and flowers of Cannabis and hemp are reported to harbour a range of fungi and yeasts (19-48, 53-73). The mechanized process of removing Cannabis flower buds from stems after harvest disrupts the stem tissues, which can release spores of endophytes, resulting in a build-up of air-borne propagules (19-48, 53-73). Up to 17 species of *Penicillium* were identified on commercially dried Cannabis buds (19-48, 53-73). These endophytes can also be problematic during tissue culture of Cannabis as surface sterilization methods do not eliminate many of them and they can continue to grow on nutrient-rich media and inhibit explant growth (1-48, 53-73). Endophytes have been shown to be problematic in tissue culture experiments with other

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plant species (1-48, 53-73). The development of tissue culture methods for Cannabis and hemp has shown some success with regard to regeneration of shoots from nodal explants for micropropagation (1-48). While several commercial companies are now producing plantlets, and this approach needs to be augmented (1-48).

Isolation and Fungal Pathogen Cultures

The use of Medical *Cannabis sativa* L. (drug or marijuana) has increased immensely over the past decade (1-48). According to the study conducted by Jerushalmi et al., (2020) in Israel (43), and Punja in Canada (2021) (19), described the first step as the identification of the fungal disease in the Cannabis crop particularly in the indoor cultivation of Medical *Cannabis sativa* L. (drug or marijuana) (19-48, 53-73). The infected inflorescence and other plant material should be collected carefully and transferred to laboratory for the isolation of the fungus and maintained the pure cultures of the fungal pathogen (19-48, 53-73). The potential pathogenic fungi were isolated from different symptomatic tissues, including inflorescences, leaves, stems, and roots, in the laboratory (19-48, 53-73). Samples were numbered, plant material was documented, and details of samples were recorded (19-48, 53-73). The isolation of fungi from the infected plants was performed by sectioning 1X1 cm pieces of relevant infected tissues and surface sterilized by immersing plant material in 70% ethanol for 30s, followed by 1% sodium hypochlorite (NaClO) for 3 min (19-48, 53-73). Thereafter, plant tissues were dried on sterile paper towels, placed aseptically on Petri plates containing PDAC 1.2% medium (Potato dextrose broth (PDB): 24 g/L; agar- 12 g/L; chloramphenicol-0.25 g/L), and kept at room temperature and allowed the fungi to develop (19-48, 53-73). After 3–5 days, fungal mycelia growing from affected plant material were transferred to new Petri plates containing PDAC 1.5% medium, and allowed to develop for another 3–5 days (19-43).

In another method, the infected plant material is cut into small pieces and surface sterilized with 70% ethanol for 30 s, followed by 1% sodium hypochlorite (NaClO) for 3 min. After sterilization, the small pieces were cultured on the Petri dishes containing Potato Dextrose Agar Medium with agar (20 g/L) and incubated at room temperature for the growth of fungus. Both pour plate method and streke methods of cultures were applied for the isolation and maintenance of pure cultures of fungal strains as developed by **Malabadi, 1994, Malabadi and Raghvendra, (1994, 1995, 1998) for the isolation of yeasts and fungi** (49-52).

Koch Postulate Assay

According to the study conducted by by Jerushalmi et al., (2020) in Israel (43), and Punja in Canada (2021) (19), the fungal species selected for **Koch Postulate Assays** were based on several criteria (19-43, 44-48, 53-73). *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani*, and *Fusarium oxysporum* were chosen, since they were the most common fungi isolated from affected plant tissues in Israel and Canada (19-43, 44-48, 53-73). To complete Koch Postulate Assays, Jerushalmi et al., (2020) in Israel (43) cultured the fungi on PDAC (Potato Dextrose Agar) 1.5% plates (19-43). After approximately one week, when the mycelium had completely covered the plate, 1 cm diameter sections were removed from the leading edge of the cultures and inoculated onto healthy Medical *Cannabis sativa* (drug or marijuana) tissue, i.e., fungi isolated from naturally infected Medical *Cannabis sativa* (drug or marijuana) tissue leaves were inoculated onto healthy leaves and inflorescences (19-43, 44-48, 53-73). The inoculation of intact plants was conducted by spraying a spore suspension of 10^6 spores/mL for each tested isolate (19-43, 44-48, 53-73).

During the **Koch Postulate Assays,** fifteen mL of sterile distilled water was added to PDAC (Potato Dextrose Agar) 1.5% plates containing the tested fungus, and spores were then gently removed using a sterile rod (19-44). The liquid was then filtered through four layers of sterile gauze and collected in a 50 mL Falcon tube (19-44). The supernatant was drained, and the pellet was suspended in 20 mL of sterile distilled H_2O, centrifuged, and dried again (19-44). According to the method reported by Jerushalmi et al., (2020) in Israel (44), spore suspensions were sprayed on healthy Medical *Cannabis sativa* (drug or marijuana) plants at the beginning of inflorescence development (inflorescence color transparent white) until run-off and the plants were then covered with a moist plastic bag for 3–5 days (19-43, 44-48, 53-73). Plants were then examined for fungal growth and disease development (19- 43). Inoculated plant tissues were placed on top of a sterile plastic net in 90 mm sterile Petri plates containing sterile paper towels soaked in 1 mL sterile distilled H_2O, sealed with Parafilm, and kept at room temperature for 3–5 days until disease symptoms developed (19-43, 44). Plates were examined for fungal growth and disease development, and the casual agents of disease were re-isolated from diseased tissue (19-43, 44-48, 53-73).

Fungal Isolation by Q-Tip Swab Method

According to the study reported by **Punja and Scott** (2023) (57), the isolation and the presence of epiphytic fungi on the surface of Cannabis inflorescences was determined by a swab method using sterile Q-tips (57). Briefly, Q-tips were gently swabbed over the surface of the inflorescence tissues consisting of aggregated pistils and immediately transferred to a Petri dish containing potato dextrose agar with 140 mg/L of streptomycin sulfate (PDA + S) (57). The Q-tip was wiped across the surface of the agar in a zigzag pattern and discarded (57). A minimum of 10 inflorescences were included each time, selected at random (57). This procedure was developed and reported by Punja and Scott (2023) (57) during September 2019–December 2021, to

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

include at least 60 such sampling times, each with 10 inflorescences sampled from the two greenhouse facilities, for a total of approximately 600 Petri dishes (57). This study also reported the sampling time varied during the year depending on the availability of the maturing inflorescences in each greenhouse (57). In addition, approximately 20 sampling times were performed on harvested and dried Cannabis inflorescences that were swabbed in a similar manner (57). For the outdoor location, approximately 20 sampling times were performed on inflorescences towards harvest in each of two growing seasons from one facility (57). The total number of Petri dishes was approximately 1000 for this study (19-48, 57). All Petri dishes were transported to the laboratory where they were incubated under ambient conditions (21°C–24 °C) with 10–14 h/day of florescent lighting for 5–7 days (57). After that time, each dish was examined for the presence of fungal colonies and morphologically distinct colonies were recorded and transferred to PDA + (57). After 2 weeks, they were subcultured again to ensure purity prior to identification (57). The swab method of sampling likely recovered a portion of these fungi that were present as viable epiphytes (57).

Identification of fungal pathogens by Molecular Methods

Fungal and oomycete pathogen identification to genus level can be achieved using morphological criteria followed by species identification by molecular methods (19-48, 53-73). To identify each morphologically unique colony to genus and species level, a PCR method utilizing primers for the ITS1-5.8S-ITS2 region of ribosomal deoxyribonucleic acid (DNA) was used by Punja and Scott (2023) (57). The isolated fungi were morphologically identified and characterized using light microscopy, and further verified using molecular methods by polymerase chain reaction (PCR) amplification (19-48, 53-73). For molecular analyses, DNA was extracted from all the putative pathogens (19-43, 44-48, 53-73). Fungal isolates were re-cultured in liquid FMM medium at 25^0 C for a week, in order to harvest dried mycelium (19-44). For Cannabis and hemp pathogen identification, the most widely used method is the polymerase chain reaction (PCR) of the ribosomal DNA region that includes the internal transcribed spacer (ITS) and intergeneric spacer regions (IGS) (19-44). PCR was used to identify most of the species (19-44, 45-48, 53-73). In addition, the elongation factor 1 (EF-1) region was used to discriminate among Fusarium species (19- 44, 45-48, 53-73). The resulting sequences were compared to the corresponding ITS1–ITS2 sequences from the National Centre for Biotechnology Information GenBank database to confirm species identity using only sequence identity values above 99% (19-44, 45-48, 53-73). A total number of 100 fungal colonies was analyzed by the study conducted by Punja and Scott (2023) (57). For *Golovinomyces* causing powdery mildew and Botrytis species causing bud rot, additional molecular markers are required to differentiate between species (19-48, 53-73). Bacterial plant pathogens have also been identified based on PCR methods that utilize the 16S region of ribosomal RNA (rRNA) in addition to other methods (19- 44, 45-48, 53-73).

In another study, **Punja and Scott** (2023) (57) investigated the diversity of fungal species present on inflorescences as epiphytes and in stem tissues as endophytes in flowering plants of Cannabis grown organically in British Columbia, Canada during 2019–2021 (19-48, 53-57, 58-73). Fresh and dried inflorescence samples were obtained at various times during production while stems were obtained at harvest (57). Fungal species in the air were assessed by exposing Petri dishes containing potato dextrose agar (PDA) + streptomycin sulfate for 1 h in the growing environment while soil samples were dilution-plated to assess soil fungal diversity (57). Colonies were identified from PCR-derived sequences of the ITS1-5.8S-ITS2 region of ribosomal deoxyribonucleic acid (19-48, 53-73). Twenty-nine species in 26 genera were recovered from inflorescences and 17 species in 11 genera originated from stem tissues (57). Approximately 96% of species found on Cannabis inflorescences were present in air and 45% were present in organic soil (57). This study demonstrated that the fungi comprised plant pathogens, saprophytes, and opportunistic human pathogens (14, 19-57). A large proportion of the species found in air and soil in organic facilities are present on Cannabis inflorescences, where they might increase total colony forming units and negatively affect product quality (19-48, 57). Some species could contribute to allergies or secondary infections in humans (1-57, 58-73).

Therefore, the results from this study indicated that one potential challenge to growing Cannabis under organic production systems is the higher prevalence of fungal spore populations in the air, whose origins are the microbially active growing substrates and components contained therein (19-48, 53-73). This is similar to the finding that unpasteurized Cocofibre used in hydroponic cultivation can also harbour a range of microbes that can potentially contaminate the stems and inflorescences of growing cannabis plants (19-48, 57-73). Several pathogens, such as *Fusarium* and *Pythium* species, cause a large number of diseases on hydroponically grown cannabis in greenhouses (57). Among them, *Penicillium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Rhizopus*, and *Botrytis* were the most common (19-48, 53-73). The diversity of fungal genera present on inflorescences in the organic greenhouse production facilities, as determined in this study, was more extensive than that previously reported from conventional production facilities (16 genera in organic vs. 10 in conventional) (19-48, 57-73).

Bacterial and Viral infections of Cannabis

The members of the genera *Pseudomonas*, *Xanthomonas* and *Pectobacterium* will be the most commonly occurring bacterial pathogens infecting Cannabis and hemp cultivation (19-48, 53-73). There are few viruses currently reported on Cannabis but this is likely due to a lack of research efforts (19-48, 53-73). Many of the recently reported viruses on hemp are reported to be seed and vector transmitted, adding an extra layer of complexity with regard to disease management (19-48, 53-73). Certified

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

laboratories perform a set of microbial isolations to enumerate total culturable yeast and mold (TCYM) and total bacteria, as well as coliforms, expressed as colony-forming units (cfu) (19-48, 53-73). There are recommendations that molecular approaches utilizing quantitative PCR (q-PCR) would be more informative for microbial determination on Cannabis inflorescences compared to plating assays (19-48, 53-73).

Fungal Disease Management Strategies

The common disease management strategies are-remove and destroy infected plants (19-48, 53-73). Irradiate dried buds with gamma or electro-beam radiation (19-48, 53-73). Another method is apply biological control agents at rooting and vegetative stages of growth (19-48, 53-73). Sustainable disease management approaches include establishing clean planting stock, modifying environmental conditions to reduce pathogen development, implementing sanitation measures, and applying fungal and bacterial biological control agents (19-48, 53-73). Vegetative cuttings should be disease-free (19-48). Irradiate leaves for 3–4 s with UV-C light daily (19-48). Apply weekly treatments of **potassium bicarbonate** (19-48). Grow strains that are tolerant to infection (19-48). Vaporize sulfur at night (19-48, 53-73). Dried Cannabis products must have minimal contamination of the inflorescences (buds) by fungi, yeasts and bacteria, as well as by specific coliform bacteria, chemical pesticides and mycotoxins (19-48, 53-73). Products failing to meet the minimum threshold requirement for these contaminants cannot be sold in the market (14, 19-48). The greatest challenge facing Cannabis and hemp producers is the management of insect pests and pathogens that attack the roots, leaves and inflorescences (19-48, 53-73). The Canadian Pest Management Regulatory Agency (PMRA) and the US Environmental Protection Agency (EPA) have approved only two chemical pesticides that producers may legally apply to their crops at the present time (19-48, 53-73). In Canada, **vaporized sulfur** is permitted indoors, while in the USA, **potassium salts of fatty acids** are registered as a pesticide (19-48, 53-73). Consequently, efficacy data on any other fungicides are lacking. Fortunately, bio-pesticides, such as potassium bicarbonate and hydrogen peroxide which have been approved and are registered for use on hemp and Cannabis (19-48, 53-73).

Fungicides that target fungal spore germination is to prevent initial infection or symptom development are widely available for most agricultural crops (19-48, 53-73). Most are applied at multiple times during the season and safety data have been generated (19-48, 53-73). For Cannabis or hemp, however, no fungicides are currently registered for use although vaporized sulfur can be used effectively to minimize establishment of powdery mildew under greenhouse conditions (19-48, 53-73). Fungicides with active ingredients that include metalaxyl, strobilurins, fludioxonil, fluopyram and pyrimethanil can provide effective control of the most important emerging pathogens (*Pythium*, *Fusarium*, *Botrytis* and powdery mildew) (19-48, 53-73). Applications of potassium bicarbonate (MilStop) sprays at weekly intervals were shown to reduce powdery mildew development on Cannabis plants (19-48, 53-73). Application of a plant extract (Regalia Maxx) from the noxious weed giant knotweed (*Reynoutria sachalinensis*) also reduced powdery mildew development on Cannabis (19-48, 53-73). This product is registered for use on Cannabis in Canada and is reported to induce disease resistance when applied to a range of plant species prior to the initiation of infection since it has less activity when used post infection (19-48, 53-73). The knotweed extract has been reported to reduce powdery mildew development on cucumber, tomato, squash and wasabi plants (19-48, 53-73). Approaches to reduce survival of pathogen propagules should emphasize sanitation measures, such as removal and destruction of all plant materials and debris that could contain inoculum of pathogens such as *Fusarium*, *Botrytis* and *Sclerotinia*, and disinfecting surfaces used during Cannabis cultivation, e.g. propagation benches, with hydrogen peroxide, UV sterilization and other disinfectants, e.g. didecyl dimethyl ammonium chloride, to reduce inoculum carry-over (19-48, 53-73).

Under field conditions, inoculum survival can be reduced through the implementation of sanitation measures such as removal and destruction of diseased plants, burial of diseased tissues deep in the soil and crop rotation (19-48, 53-73). Indoor climate management to provide dry conditions is recommended to reduce *Botrytis* bud rot, since reduced relative humidity and moisture deposition on the inflorescences can reduce spore germination and infection (19-48, 53-73). Under field conditions, *Botrytis* bud rot can be a devastating disease under cool and wet weather on Cannabis and hemp (19-48, 53-73). Removal and destruction of diseased inflorescences and early harvest are currently practiced by licensed producers to reduce the potential for spore production and pathogen spread (19-48, 53-73). These physical methods can minimize disease outbreaks but are labour-intensive (19-48, 53-73). Reproduction of these pathogens on diseased tissues can further added to the inoculum load and lead to further spread within a Cannabis growing facility (19-48, 53-73). Sanitization methods to ensure that introduction and spread of pathogens within a Cannabis growing facility are minimized are needed (19-48, 53-73). Foliar pathogens such as powdery mildew and *Botrytis* bud rot can similarly spread as air-borne inoculum or through vegetative propagation (19-48, 53-73). Both of these pathogens are known to reduce growth and quality of Cannabis plants, and disease management is difficult (19-48, 53-73). In the case of *Botrytis*, infection of inflorescences during production can lead to significant post-harvest losses during storage (19-478, 53-73).

Air-borne saprophytic molds that end up on Cannabis inflorescences different *Penicillium* species (19-48, 53-73). In addition, *Botrytis* bud rot can pose challenges to producers during production and also as a post-harvest problem (19-48, 53-73).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

Most of the root-infecting pathogens are not visibly detrimental to plant growth unless infection occurs early (19-48, 53-73). However, destruction of roots can result in as-yet undetermined reductions in yield and quality (19-48, 53-73). Powdery mildew infection is commonly present in the most production facilities and will require proactive management methods and potential identification and utility of disease-resistant genetic selections (19-48, 53-73). The response of different Cannabis strains (genotypes) to the various pathogens identified and reported by Punja et al., 2019 (19-48) is an important aspect of disease management (19-48, 53-73).

Pesticide Problems in Cannabis Industry

Pathogens are a pain in the neck of every breeder (42). They affect the quality and quantity of yield, thus defeating the aim of cultivation (19-48, 53-73). Plant pathogens infecting marijuana (*Cannabis sativa* L.) plants reduce growth of the crop by affecting the roots, crown, and foliage (19-48, 53-73). In addition, fungi (molds) that colonize the inflorescences (buds) during development or after harvest, which colonize internal tissues as endophytes, can reduce product quality (19-48, 53-73). The pathogens and molds that affect *Cannabis sativa* L. grown hydroponically indoors (in environmentally controlled growth rooms and greenhouses) (19-48, 53-73). Molds are defined as fungi present on living or dead plant materials that are not associated with disease symptoms and may be present as incidental contaminants in the air or on growing substrates, or be part of the succession of microbes that decompose plant materials (19-73). These pathogens and molds were found to occur on Cannabis plants during cultivation in greenhouse and indoor controlled environment growing facilities as well as in outdoor field environments (19-48, 53-73). On root systems of Cannabis plants, pathogens that included species of *Fusarium* and *Pythium* caused browning and decay on roots that resulted in stunted growth, yellowing, and sometimes death of the affected plants (19-73). Up to four species of *Pythium* and three of *Fusarium* were identified (19-73).

The process of mechanical trimming of Cannabis buds after harvest (wet trim) and the associated wounding of the tissues caused an observable increase in the recovery of *Penicillium* and *Cladosporium* colonies compared to untrimmed harvested buds, indicating their populations on the surface of tissues were increased (19-48, 53-73). Wounding is known to increase the colonization of a range of fruits by *Penicillium* after harvest (19-55-73). Exudation of nutrients from cut tissues would have enhanced the proliferation of these opportunistic molds (19-73). In addition, internally borne mold spores e.g., in the pith could have been released through wounding of tissues and become air-borne (19-73). *Cladosporium* is commonly found in indoor environments (56) and was the most commonly identified mold, especially in the summer (56). Management of these molds on Cannabis buds would required careful handling and drying and storage under conditions that discourage their further proliferation (19-48, 53-73). The fact that they are so ubiquitous outdoors and indoors, and are prolific spore producers, as well as are harboured internally, provides additional challenges to producers aiming to achieve a high-quality, minimally contaminated product (19-56).

The diversity of Cannabis-associated *Aspergillus, Cladosporium, Fusarium, Mucor,* and *Penicillium* molds as well as their harmful toxins (e.g., aflatoxins, fumonisins, ochratoxins, trichothecenes, and T-2 toxins), are but the tip of the iceberg (19-73). These molds are spore-producing generalists, and can spread by air as a vehicle with significant potential for distribution across production regions and continents (19-48, 53-73). **Aspergillus** is a mold that produces extremely hardy spores and is capable of rapid replication (r-strategists) at much lower water and nutrient levels than most microorganisms (19-73). A. flavus, A. niger, and A. parasiticus are molds linked to the Cannabis host that may be particularly hazardous to asthma patients (19-73). According to one of the study, gardeners and farmers in particular are believed to inhale thousands of Aspergilli spores every day (19-73). It is noteworthy that pulmonary aspergillosis can be hard to diagnose and treat—especially invasive aspergillosis, for which the mortality rate is quite high, which calls for preventing the establishment of molds in Cannabis production systems (19-73). The USDA list of fungi indicated that Cannabis is also host for *Trichoderma* and *Trichothecium* (T. roseum), potential biological control agents (BCA). However, such generalists also pose important health risks (19-73).

As the need for Cannabis products skyrocket, farmers face the dilemma of n December 2022, The Oregon Liquor and Cannabis Commission (OLCC) reported the recall of 9,300 products still on sale and 13,600 already purchased due to possible pesticide contamination (42). There were also traces of mold and fungus growth due to high levels of moisture after packaging (14, 42-73). A 2019 research showed that diverse pesticides directly or indirectly pollute the air, water, soil, and ecosystem, leading to health hazards (14, 42-73). According to the WHO, pesticides are among the leading cause of death by self-poisoning, especially in low- and middle-income countries (14, 42). In 2015, about 30,000 packages of marijuana-infused edibles were recalled in Colorado due to potential pesticide contamination (14, 42). HPLV, molds, and fungi have continued to plague Cannabis farms, with farmers seeking solace in pesticides that leave residues in the plants (19-42, 53-73). These pesticide-contaminated plants or products serve as a potential route of pesticide exposure to patients with neurological diseases, thus worsening their burdens and outcomes (14, 19-42, 53-73). The Cannabis industry has a pesticide problem with a simple environmental solution. Every year, millions of dollars are lost to pathogens and their activities on cannabis farms (14, 42). These

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

huge losses affect pre-harvest and post-harvest operations (42). The most prominent of these challenges is product recalls which can be avoided with the right environmental solution to common contaminants like molds, pesticides, fungi, and bacteria (14, 42).

Pesticides have been found in all Cannabis products, from flowers to edibles, vapes, and smokes (14, 42, 53-73). Information from research showed that rather than alleviating a patient's condition, pesticide-contaminated Cannabis harms the patients and exposes them to more adverse effects (14, 42-73). Another point is that the **Cannabis plant acts as a sponge** that sucks up compounds from the soil (42). This is why some researchers are considered Cannabis plant possibly used in cleaning polluted soils (42). So far, researchers have discovered the potential benefit of Cannabis in bioremediation and cleaning up soils contaminated by fertilizers, pesticides, and heavy metals like cadmium and lead (42).

The pesticide pandemic in the Cannabis industry needs urgent attention and calls for a need to abandon the archaic, harmful chemicals for processes that protect the plants by focusing on "the source of infection rather than dealing with the infection after it has manifested in the plant" (14, 42). The pesticide problem in the Cannabis industry stemmed from the need to keep the plant free from pests, fungi, bacteria, and molds, which can be prevented by maintaining an aseptic growing environment (42). It is pertinent to note that pesticides will not be needed when plants are grown in a conducive and aseptic condition (42). They are now focused on eco-friendly solutions that deal with pathogens without compromising product quality or consumer and environmental safety (19-48).

So Safety Net offers a range of products and services that have been proven effective in various agricultural-related areas that take care of contamination from the source (42). Their products and services focus on environmental cleaning, disinfection and protection products, UV disinfection products, air purification products, and water purification solutions (42). Safety Net's eco-friendly products have the potential to eliminate the need for pesticides and give farmers a chance to save more with less costly products while dealing with pathogens with ease (42). With Safety Net's Bio-security program, farmers can enjoy larger, healthier plants with the potential for more yield, which means higher profit margins (42). When compared annually, they offer a process where the cost of products to clean, disinfect and protect the growing environment is less than the cost of pesticides and other growth enhancement products (42). The products at Safety Net America have a broad use case, as they can be applied to any form of agriculture currently suffering from the pesticide pandemic (42). So far, the products of Safety Net have shown good results in Cannabis, wine growing, food packaging, chicken house, and much more (42).

With Safety Net's Bio-Security program, Cannabis farms will be safeguarded from pathogens while maintaining your product's compliance and safety (42). Safety Net's Bio-Security program provide products and services designed to safeguard environments beyond the normal approach to disinfecting (42). They are not only the trusted partner to address the needs of Cannabis business today but the partner to trust in developing solutions for the needs of tomorrow in the rapidly evolving cannabis industry (42).

Plant Disease Management by Artificial Intelligence

Crop diseases are one of the main challenges in the farming sector. Thus, there is a need to identify crop diseases at the earliest stage to lessen disease severity and to curb disease propagation on agriculture farms (74-115). The management of large-scale Cannabis production necessitates a variety of timely actions, such as keeping an eye out for diseases which limits them to unwanted items (19-74). The most common causes of Cannabis disease are insect pests, bacteria, viruses, algae, and fungi (19-48, 53-73). Certain Cannabis diseases have no visual indications. Therefore, advanced analytical methods are used in these cases (19-74). Most of the Cannabis that is used recreationally and medicinally is grown at high density in greenhouses or warehouse settings (19-48, 53-73). The cultivation of Cannabis in such a manner resulted in high incidences of infection with fungi, bacteria, viruses, and nematodes (19-48, 53-73). All production environments face challenges from plant pathogens. However, in some cases greenhouse systems share more diseases than outdoor in the Cannabis industry (19-48, 53-73). Therefore, the ability to control this infection is very essential. Plant pathogens that infect marijuana plants reduce crop growth by affecting the roots, crown, foliage, leaves, stems, and inflorescences (19-73). Fungus is the main group of Cannabis pathogens that colonizes inflorescences during development or after harvest, in addition to internal tissues like endophytes (19-73). More importantly, the extensive development of fungi such as *Botrytis cinerea, Fusarium* and *Penicillium* in the inflorescences can lead to the accumulation of mycotoxins in the tissues; this causes additional health concerns for consumers (19-48, 53-73). Pathogens that directly infect the inflorescences of either crop at any time during their development have the potential to significantly reduce the economic value of the harvested product (19-48, 53-73). These 'bud rot' pathogens are reported to include *Botrytis cinerea,* a widespread and destructive pathogen of Cannabis and hemp as well as several species of *Fusarium* and *Penicillium* that can cause pre- and postharvest bud rots (19-48, 53-73). *Botrytis cinerea* is the most widespread generalist species, causing grey mould on over 1400 plant species and it is commonly found in both agricultural and non-agricultural environments and is prevalent in greenhouses (19-48, 53-73).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

However, the most of the infected Cannabis have visible signs, and an experienced plant pathologist identifies the disease by examining infected Cannabis leaves, inflorescence, stem, and root using an optical microscope (19-73). An accurate Cannabis disease diagnosis required good observation skills and knowledge to recognize precise symptoms of a certain disease (19-73). The manual Cannabis disease identification process is time-consuming and dependent on the availability of experienced plant pathologists (19-73). Moreover, continual Cannabis plant monitoring is required, which is highly expensive when dealing with large farms (19-73). Furthermore, the excessive variety of plants and variations in symptoms over time due to climate changes even the experienced pathologist may unable to accurately identify certain diseases and may take a long time (19-74, 75-115). For sustainable and correct agriculture, as well as to avoid unnecessary waste of financial and other resources, timely and precise identification of plant diseases is critical (74-115).

Manual identification of crop diseases is both fastidious and inaccurate, meaning it is only feasible in small farms (74-115). In contrast, automatic disease detection is significantly more accurate and takes less time and labour (74-115). Disease attacks are constant threats to Cannabis agriculture and cause heavy losses in the country's economy. Therefore, early detection can mitigate the severity of diseases and protect crops (74-115). However, manual disease identification is both time-consuming and error prone, and required a thorough knowledge of plant pathogens. Instead, automated methods save both time and effort (74-115). Therefore, early identification of diseases is crucial to avoid huge losses and reduce the excessive use of pesticides, which can harm human health and the environment (74-115). In most cases, and especially in developing countries and small farms, farmers identify crop diseases with the naked eye based on visual symptoms (74-115). This is a tedious task that requires expertise in plant pathology and excessive treatment time (74-115). Moreover, if the field is attacked by a rare disease, farmers seek expert advice to obtain an accurate and efficient diagnosis, which obviously generates additional treatment costs. (74-115). In this regard, several methods have been proposed to automate the process of disease detection (74-115). These methods for automatic recognition of crop diseases are divided into two groups, direct and indirect methods. Direct methods comprise molecular and serological techniques that provide accurate and direct detection of the pathogens triggering the disease, although these techniques required a significant amount of time for the collection, processing, and analysis of the collected samples (74-115). By comparison, optical imaging techniques are among the indirect methods that are able to identify diseases and predict the health of the crop through different parameters such as morphological change and transpiration rate (74-115). Fluorescence and hyperspectral imaging are some of the most widely used indirect methods for early disease identification (74-115).

Artificial intelligence (AI) is a broad field of computer science concerned with building smart machines capable of performing tasks that typically required human intelligence (17, 18, 118, 119). The primary goal of Artificial intelligence (AI) is to enable computers and machines to perform cognitive functions such as problem-solving, decision making, perception, and comprehension of human communication (17, 18, 118, 119). Subfields of Artificial intelligence (AI) such as Machine Learning (ML), Natural Language processing, Image Processing and Data mining have become an important topic for today's tech giants (17, 18, 118, 119). Many industries, including information technology, telecommunications, transportation, traffic management, health care, education, criminal justice, defence, banking, and agriculture, have the potential to be transformed by artificial intelligence (17, 18, 118, 119). Without compromising the significant characteristics that identify mankind, the Artificial intelligence (AI) systems are deliberate, intelligent, and flexible with adequate security (17, 18, 118, 119, 74-115). Artificial intelligence (AI), boosted through deep learning (DL), has achieved significant breakthroughs and is a powerful tool for managing large complex datasets such as the interplay between the microbiome, crop plants, and their environment (17, 18, 118, 119, 74-115). Artificial intelligence (AI) has proven capable of improving performance in many areas such as predicting earthquakes, classifying plant species based on leaf/plant images, automobile self—driving, recognizing faces, and filtering emails (74-115). ChatGPT, the most advanced natural language processing (NLP) which is Artificial intelligence (AI) model, received more than 100 million users within the first two months and receives 13 million visits a day as of 2023 (74-115). Data mining can be used to extract taxonomic composition, gene functions, and associations between plant microbiomes and host plant phenotypes (17, 18, 118, 119, 74-115). Artificial intelligence (AI) boosted by Machine learning (ML) and Deep learning (DL) advancements, is being explored as a solution to meet some of the challenges associated with data size and complexity when considering the plant microbiome in cropping systems (74-117, 17, 18, 118, 119).

The technology of Artificial Intelligence (AI) in the detection and management of disease has already been employed in Rice, tea maize, tomato, grape, turmeric (74-117). Plant disease detection and identification at the early stage of plant growth is very important using Artificial Intelligence (AI) techniques (74-117). At present, object detection algorithms based on Deep Learning are constantly being developed and adopted for plant disease localization and classification (74-117). These methods determined the precise location and class of the disease (74-115). However, for a real complex natural environment, the performance degrades. Despite recent progress, there is still a need for improvement in the application of Deep learning (DL) architectures, particularly novel Deep learning (DL) architectures for crop plant disease classification in terms of generalization robustness and identification accuracy (74-117). Moreover, the requirement for efficient models with fewer training parameters and faster training speed without compromising the performance is unavoidable (74-117). Conversational AI systems are

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

commonly known as chatbots. They are intelligent models that can be further categorized into either retrieval-based or generative models or even as a combination of both (17, 18, 74-119).

Historically, disease identification has been supported by agricultural extension organizations or other institutions, such as local plant clinics (74-117). In more recent times, such efforts have additionally been supported by providing information for disease diagnosis online, leveraging the increasing internet penetration worldwide (74-117). Even more recently, tools based on mobile phones have proliferated, taking advantage of the historically unparalleled rapid uptake of mobile phone technology in all parts of the world (74-115). Applications of machine learning (ML) and deep learning (DL) in the field of agriculture are picking up energy. Strategies of image preparing are utilized for precise discovery and grouping of harvest disease and the exact location and order of the plant disease‟s significant for the productive development of the crop (74-115).

The smartphone mobile diagnostic tool with real-time feedback comes with various benefits as follows: (i) Farmers do not have to wait for experts, as they can get instant advice on their gardens on three major crops, such as cassava, maize, and beans (74-117). (ii) The findings from this study paves way for the agricultural recommender systems in developing worlds by improving the livelihoods of smallholder farmers through early intervention measures, thus alleviating the food security problem in sub-Saharan Africa (74-115). Smart phones in particular offer very novel approaches to help identify diseases because of their computing power, high resolution displays, and extensive built-in sets of accessories, such as advanced HD cameras (74-115). The combined factors of widespread smartphone penetration, HD cameras, and high performance processors in mobile devices lead to a situation where disease diagnosis based on automated image recognition, if technically feasible, can be made available at an unprecedented scale (74-115). In order to develop accurate image classifiers for the purposes of plant disease diagnosis, a large, verified dataset of images of diseased and healthy plants are needed (74-117). Until very recently, such a dataset did not exist, and even smaller datasets were not freely available. To address this problem, the **PlantVillage** project has begun collecting tens of thousands of images of healthy and diseased crop plants, and has made them openly and freely available (74-117, 120). Currently, infectious diseases reduced the potential yield by an average of 40% with many farmers in the developing world experiencing yield losses as high as 100% (120). The widespread distribution of smartphones among crop growers around the world with an expected 5 billion smartphones by 2020 offers the potential of turning the smartphone into a valuable tool for diverse communities growing food (120). One potential application is the development of mobile disease diagnostics through machine learning and crowd sourcing (120).

Hughes, and Salathe, 2015 (120) announced the release of over 50,000 expertly curated images on healthy and infected leaves of crops plants through the existing online platform PlantVillage (120). This work described both the data and the platform. These data are the beginning of an on-going, crowdsourcing effort to enable computer vision approaches to help solve the problem of yield losses in crop plants due to infectious diseases (120). Computer vision, and object recognition in particular, has made tremendous advances in the past few years (74-115). The PASCAL VOC Challenge, and more recently the Large Scale Visual Recognition Challenge (ILSVRC) based on the ImageNet dataset have been widely used as benchmarks for numerous visualization-related problems in computer vision, including object classification (74-117).

The deep learning (DL)-based approaches are extensively being applied in different fields including agriculture as well (74-117). These techniques automatically compute discriminative features directly from the input samples, thus avoiding complicated image pre-processing and reducing the memory footprint (74-117). The Convolutional Neural Network (CNN) is a well-known Deep learning (DL) model that showed effective performance in pattern recognition and is widely employed for early plant leaf disease identification (74-115). In recent studies, CNN is primarily used for crop plant disease identification and classification (74-117). These approaches have shown promising results in disease crop-related classification tasks due to effective feature representation (74-115). The mature CNN architectures in computer vision such as AlexNet. ResNet, EfficientNet , MobileNet and Densenet are extensively used in existing plant disease categorization methods (74-117).

In recent years, due to the advancements in Artificial Intelligence (AI) technology, image-based automated process control systems are introduced that can automatically identify the diseased plants and offer valuable insight to agronomists (74-115). Automatic detection techniques assist farmers in improving crop quality while also reducing disease occurrence through early identification, timely, and appropriate treatment (74-115). Image processing is used for measuring the affected area of disease and to determine the difference in the color of the affected area (74-117). Initially, machine learning (ML)-based models were proposed for the identification and classification of plant diseases (74-115). The methods such as support vector machine (SVM), decision tree (DT), random forest (RF), and K-nearest neighbours (KNN) have been employed for early and accurate detection of crop plant diseases (74-115). The ML-based techniques are simpler to deploy and do not require huge training data. However, they are slow due to complex pre-processing and dependent on the knowledge of experienced human specialists for extraction and the selection of suitable features required to perform the classification (74-117). Moreover, selecting a large feature set increases the computing complexity, while using a small feature set degrades the identification performance (74-117).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

Therefore, the detection efficacy of these approaches depends on the quality and representation of extracted features and is susceptible to errors when working with a large amount of data (74-115). Thus, ML-based techniques have limited accuracy for automated plant disease identification (74-117). The accurate identification of several plant diseases is still challenging because the diseased spots have varying appearances, such as size, shape, hues, and position (74-115). Moreover, the presence of background noise, intra-class differences at different growth stages, and multiple tiny and dense diseased spots on the same leaf affect the diagnosis of plant leaf diseases (74-117). Furthermore, variations in illumination and brightness conditions during the image acquisition process of leaves contribute to the unsatisfactory detection results of computer-aided design (CAD) solutions (74-115). To solve the problems of existing techniques, one of the recent study used a robust **drone-based** deep learning approach (74-115). More specifically, this study introduced an improved EfficientNetV2-B4 with additional added dense layers at the end of the architecture (74-115). The customized EfficientNetV2-B4 calculated the deep key points and classifies them in their related classes by utilizing an end-to-end training architecture (74-115). The custom EfficientNetV2 model computes the deep features and classifies them in their respective class using an end-to-end training architecture (74-115). The presented framework, namely, the improved EfficientNetV2 is better than the original network in terms of detection accuracy, time, and number of model parameters (74-115). Moreover, the proposed method is more robust to unseen examples as well due to the inclusion of dense layers (74-115). The introduced approach is capable of effectively classifying the plant disease under varying challenging conditions such as the presence of blurring, noise, and variations in color, size, and position of the infected regions (74-115). For the evaluation of the plant disease detection and classification performance of the approach, Albattah et al., 2022 (74) have employed the Plant Village database (Albattah et al., 2022) (74-117). The **Plant Village dataset** is a large and online accessible standard database of crop leaf disease classification, which is extensively explored by several techniques from the past for performance assessment (74-117).

Le et al. (2020) (82) reported an approach to locate and categorize the crops and weed-based diseases (74-115). In the first step, the noise from the suspected samples was removed by employing the morphological opening and closing operation (82). In the next step, a custom model, namely, the filtered LBP approach along with contour mask and coefficient k (k-FLBPCM) was introduced to extract the key points from the enhanced image (82). Finally, the computed key points were employed for the SVM training to achieve the diseased leaf region categorization (82). The technique by Le et al. (2020) (82) showed an improved plant disease recognition power with an accuracy of 98.63% (82). However, this approach is not robust for the image containing perspective distortions (74, 82). Pantazi et al. (2019) (74, 84) presented a method to locate and categorize several crop diseases (84). In the first step, the GrabCut approach was used over the suspected image to perform the sample segmentation (74, 84). In the next step, the Hue, Saturation, and Value (HSV) transform was applied to the segmented image from which the features were computed using the LBP algorithm (74-84). Finally, the extracted key points were used to train the SVM (84). The study by Pantazi et al. (2019) (84) is effective to plant leaf affected region categorization with the accuracy of 95% (84). However, its detection accuracy degrades over the noisy samples (74-84). The approach by Dwivedi et al. (2021) (105) showed improved results to categorize several diseases of the grape plant with an accuracy value of 99.93%, however, shows poor performance for real-world scenarios (74, 105).

Ahmad et al. (2020) (81) presented a method to identify and recognize the affected areas of several plant leaves. Initially, Directional Local Quinary Patterns (DLQPs) were used for feature extraction from the input images (74, 81). The calculated key points were utilized for training the SVM classifier to classify the crop leaf diseases (81). This method of Ahmad et al., (2020) worked well for plant disease classification with the classification accuracy of 96.50% (81). However, detection performance can be further enhanced using the shape and color based information of the suspected images (74, 81). In another parallel study, Kaur (2021) (77) proposed an ML-based approach for plant disease classification (74, 77). Several feature extraction algorithms, namely, Local Binary Pattern (LBP), Gray Level Co-Occurrence Matrix (GLCM), Shift-Invariant Feature Transform (SIFT), and Gabor were applied for feature extraction from the input images. Then, the ML classifiers, namely, SVM, KNN, Artificial Neural Network (ANN), and RF were trained to accomplish the plant disease categorization task (74, 77). The study by Kaur (2021) (77) attains the best result for Gabor features with the classification accuracy of 90.23% (74, 77). However, performance needs further improvements (74, 77). Shrivastava and Pradhan (2021) (78, 74) proposed a solution for the detection and classification of different plant diseases (74, 78). Initially, the 14 color spaces were used to extract the 172 key points from the suspected samples (74-86). Then, the calculated key points were used for the training of SVM. The approach by Shrivastava and Pradhan (2021) (74-86) demonstrated improved plant leaf diseased region categorization performance with an accuracy of 94.68% (78, 74). However, this approach is not robust to samples with huge distortions (74, 78, 79). Another approach to detect and classify tea plant leaf diseases was presented in the study by Sun et al. (2019) (74-83). Initially, the Simple Linear Iterative Cluster (SLIC) was used to divide the input image into several blocks, from which the features were computed via the Harris method (74-83). Then, the convex hull method was applied to detect the fuzzy salient areas, and GLCM technique was utilized to calculate the feature vector (74, 83). Finally, the extracted keypoints were used to train the SVM classifier (74, 83). The framework (Sun et al., 2019) exhibits better crop leaf disease categorization with the accuracy of 98.50%; however, this method is economically complex (74-83).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

The study by Oo and Htun (2018) (85) exhibits enhanced crop leaf diseased region classification performance with the accuracy of 84.6% (85). However, evaluation is performed on a database with a small number of samples (74-85). Ramesh et al. (2018) (85) introduced a technique to categorize the various abnormalities of crop leaves (74-85). The Histogram of Oriented Gradient (HOG) approach was applied for keypoint computation, which was employed for the RF classifier training (74-85). The method described by Liao and Vemuri (2002) (117) and Ramesh et al., (2018) (86) showed better crop leaf disease categorization with the accuracy of 70.14% (74, 86). However, performance requires more enhancement (74-86, 117). Atila et al. (2021) (116) proposed a DL-based method, namely, the EfficientNet framework for the identification and categorization of crop diseases (74-117). The performance of the model was evaluated over both the original and augmented dataset. The approach by Atila et al. (2021) (116) performs well for plant disease classification with an accuracy of 99.97% (74, 116). However, at the rate of higher computational complexity (74, 116). Richey et al. (2020) (111) presented a lightweight approach to locate and recognize different diseases of the maize plant (74, 111). The approach ResNet50 was used for computing the features from the samples under investigation which are then categorized into relevant groups (74, 111). The approach by Richey et al. (2020) (111) gives a low-cost framework for classifying the crop diseases with the classification accuracy of 99% (74, 111). However, the work is less efficient to be employed on mobile phones due to limited power, execution, and space constraints (74-117).

Health issues related to Fungal infections of Cannabis

Cannabis can contain fungal pathogens that cause serious and often fatal infections in persons with immunocompromised conditions, such as cancer, transplant, or infection with HIV (14, 19-73). The frequency of fungal infections associated with Cannabis is unknown but is a growing concern as more countries legalized its medicinal and recreational use (14, 19-73). Characterization, prevention, and control of Cannabis pathogens, including pre- and post-harvest molds and their mycotoxins, will represent a major challenge for Cannabis science and safe product production (14, 19-48, 53-73). Contamination of Cannabis plants and products (i.e., recreational- and pharmaceutical-grades) with mycotoxigenic organisms, including species of *Aspergillus*, *Penicillium*, and *Fusarium*, pose serious challenges (14, 19-48, 53-73). Exposure to fungal spores via Medical *Cannabis sativa* (drug or marijuana) consumption may lead to potential disease complications (14-48,53-73). Another more extreme example is a case of two immunocompromised patients that inhaled Medical *Cannabis sativa* (drug or marijuana) as an analgesic and developed invasive pulmonary aspergillosis (14, 19-48, 53-73). In retrospect, it was found that the Medical *Cannabis sativa* (drug or marijuana) supplied to these patients was contaminated with spores of *Aspergillus* spp., which was likely the source of infection (14, 19-48, 53-73). Furthermore, air samples from the lungs of workers from Medical *Cannabis sativa* (drug or marijuana) farms have contained a significantly higher than normal concentration of microorganisms, especially different kinds of *Ascomycota*, of which *Botrytis cinerea* was the most common (14-48, 53-73). Medical *Cannabis sativa* (drug or marijuana) pathogens imposed a direct health hazard to patients that consumed infected inflorescences (14, 19-48, 53-73). For example, *Botrytis cinerea*, which is one of the most common pathogens of Medical *Cannabis sativa* (drug or marijuana) is a known allergen and can lead to harsh reactions in humans (14, 19- 48, 53-73).

The presence of certain opportunistic fungi that can potentially affect humans, however, may necessitate more care in providing workers with necessary protection from inhalation of spores within organic Cannabis facilities (19-48, 53-73). Furthermore, the higher fungal spore populations in organic greenhouse facilities could lead to a higher concentration of fungal spores present in inflorescences that could cause products to fail to meet quality assurance criteria (14, 19-48, 53-73). The detection of *Scedosporium aurantiacum* in an Cannabis organic greenhouse production facility could be of potential interest as it belongs to the group of fungi that has recently emerged as an aetiologic agent of localized and disseminated diseases in both immunocompromised and immunocompetent humans (19-48. 53-73). This ascomycete fungus has been recovered from soil, sewage, cattle, and poultry manures (14, 19-73). Consistently, *Scedosporium aurantiacum* is considered as the second most frequent filamentous fungal genus (after *Aspergillus*) to colonize the lungs of patients with cystic fibrosis (14, 19-73). This fungus can cause invasive infections in transplant recipients and patients with haematological diseases, resulted in a progressive and severe deterioration of lung function of these individuals over time (19-48, 53-73).

Furthermore, the presence of pathogens and mycotoxins are expected to rise together with increased demands for Cannabis production at a large scale, under both greenhouse and field conditions (14, 19-48, 53-73). The problem related to specific molds (i.e., *Aspergillus* and *Penicillium*) and high concentration of mycotoxins and toxic pesticide residues is amplified by greenhouse/closed and relatively humid environmental conditions (14, 19-48, 53-73). In many instances, the open agricultural fields are rather exposed to climate change-associated phytopathogenic and mycotoxigenic fungi and infectious bacteria (14, 19-73). In both cases and environments, unhealthy biological and chemical contaminants in Cannabis samples and/or commodities may induced serious physical, mental, behavioural, and social health consequences in humans, in the event that proper preventative measures are not taken (14, 19-73). A recent UC Davis Medical Center report authored by W. Walker based on DNA analyses found that >20% of tested medical Cannabis samples were contaminated with a range of dangerous bacteria and fungal molds (14, 19-73). Thus, even Cannabis labelled as medicinal-grade could pose a dangerous and potentially lethal threat to human beings, especially in vulnerable population sub-groups with suppressed immune systems (e.g., in context of cancer,

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume VIII Issue VIII August 2023

transplant recipients, and HIV/AIDS) or in the elderly, where Cannabis is increasingly used for pain/mood management and for inducing sleep (14, 19-73).

Fungal contamination has been highlighted in several case reports of lung infections, including from *Aspergillus*, which frequently occurs on Cannabis plants (14, 19-73). Cannabis inflorescence buds are often contaminated with molds and mycotoxins, particularly if not stored properly (14, 19-48,53-73). Methods for controlling mycotoxins are mostly preventive during production, handling, transportation, storage, and processing (14, 19-73). Two of the main types of mycotoxins associated with contaminated Cannabis products are **aflatoxins** and **ochratoxins** (produced by *Aspergillus flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*) (14, 19-73). Smoked marijuana contaminated with aspergilla have developed clinical, laboratory, and radiologic findings consistent with invasive pulmonary—and allergic broncho-pulmonary aspergillosis (14, 19-73). In addition, the accumulation of aflatoxins can also cause lung and liver cancer and can cross the placental barrier to exert harmful effects in the fetus (14, 19-73). Ochratoxins, such as those produced by *Aspergillus ochraceous*, have a similar mutagenic and carcinogenic profile to aflatoxins (14, 19-73). Aflatoxins, but not fumonisins (produced by *Fusarium* species) nor ochratoxin A, are tested under the Canadian ACMPR program (14, 19-73). While full mycotoxin testing of Cannabis is possible, thresholds for toxicity have not been established representing future challenges for Cannabis and cannabinoid sciences (19-48, 53-73). Case reports describing the effect of smoking, vaping, or inhaling aerosolized contaminated marijuana demonstrated some of the graver risks to patients, especially those with leukemia, lymphoma, AIDS, or those with medical conditions requiring immune-suppressing therapies (14, 19-73).

Conclusion

To date, there are over **88 known fungal species** affecting Medical *Cannabis sativa* (drug or marijuana) and hemp plants at all the growth stages, not including storage pathogens. Some of the fungal pathogen that can attack Cannabis crops are *Botrytis*, *Alternaria*, *Fusarium*, *Penicillium*, *Cladosporium*, and *Aspergillus*. Fungal diseases are Powdery Mildew, Damping off, and Mildew. Of these fungal pathogens, the most common inflorescence disease is gray mold, caused by *Botrytis cinerea*. Therefore, fungi are nightmare for Cannabis industries and determined to ruin the whole crop. However, manual disease identification is both time-consuming and error prone, and required a thorough knowledge of plant pathogens. Instead, automated methods save both time and effort. Therefore, early identification of diseases is crucial to avoid huge losses and reduced the excessive use of pesticides, which can harm human health and the environment. More importantly, the extensive development of fungi such as *Botrytis cinerea*, *Fusarium* and *Penicillium* in the inflorescences can lead to the accumulation of mycotoxins in the tissues which causes additional health concerns for consumers. Cannabis can contain fungal pathogens that cause serious and often fatal infections in persons with immunocompromised conditions, such as cancer, transplant, or infection with HIV. Furthermore, the presence of pathogens and mycotoxins are expected to rise together with increased demands for Cannabis production at a large scale, under both greenhouse and field conditions. Fungal contamination has been highlighted in several case reports of lung infections, including from *Aspergillus*, which frequently occurs on Cannabis plants.

The technology of Artificial Intelligence (AI) in the detection and management of disease has already been employed in rice, tea maize, tomato, grape, and turmeric. Plant disease detection and identification at the early stage of plant growth is very important using Artificial Intelligence (AI) techniques. In recent years, due to the advancements in Artificial Intelligence (AI) technology, image-based automated process control systems are introduced that can automatically identify the diseased plants and offer valuable insight to agronomists. Automatic detection techniques assist farmers in improving crop quality while also reducing disease occurrence through early identification, timely, and appropriate treatment. Initially, machine learning (ML)-based models were proposed for the identification and classification of plant diseases.

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