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Organically grown cannabis (*Cannabis sativa* L.) plants contain a diverse range of culturable epiphytic and endophytic fungi in inflorescences and stem tissues

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Abstract

We 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 during 2019–2021. Fresh and dried inflorescence samples were obtained at various times during production while stems were obtained at harvest. Fungal species in the air were assessed by exposing Petri dishes containing potato dextrose agar + streptomycin sulfate for 1 h in the growing environment while soil samples were dilution-plated to assess soil fungal diversity. Colonies were identified from PCR-derived sequences of the ITS1-5.8S-ITS2 region of ribosomal deoxyribonucleic acid. Twenty-nine species in 26 genera were recovered from inflorescences and 17 species in 11 genera originated from stem tissues. Approximately 96% of species found on inflorescences were present in air and 45% were present in organic soil. The fungi comprised plant pathogens, saprophytes, and opportunistic human pathogens. A large proportion of the species found in air and soil in organic facilities are present on cannabis inflorescences, where they may increase total colony forming units and negatively affect product quality. Some species could contribute to allergies or secondary infections in humans. The potential benefits of endophytes within organically grown cannabis plants remain unexplored.

Key words: *Cannabis sativa*, post-harvest quality, soil fungi, organic production, plant pathogens, endophytes

Introduction

Large-scale production of THC-containing *Cannabis sativa* L. (referred to here as cannabis) for medicinal and recreational use is currently permitted in Canada following its legalization in 2018. Licensed producers have several options with regard to the production methods they use. Plants can be grown indoors in controlled environment rooms, in greenhouses, as well as outdoors under natural field conditions. Hydroponic cultivation is 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. Alternatively, producers may elect to grow cannabis plants in soil under conditions that can qualify as "organic", which requires that fertilizers, pest control products, and other materials used during production must meet the requirements to be considered organic. At present, in North America, federal regulatory agencies such as Health Canada and the U.S. Department of Agriculture have not established specific guidelines for cannabis producers to follow to meet organic certification standards at the national level. Therefore, producers must rely on other regulatory agencies to guide them on the requirements to satisfy organic certification. In British Columbia (BC), where the current study was conducted, these include organizations such as the Fraser Valley Organic Producers Association, the Pacific Agricultural Certification Society, and Pro-Cert. Presently, five licensed producers have chosen to grow their cannabis crops to meet organic certification standards in BC.

Worldwide, numerous crop plants are grown organically and there is a vast literature on the pros and cons of organic methods of production (Reganold and Wachter 2016). However, there have been no prior studies conducted to assess how organically produced cannabis plants compare to the more commonly used hydroponic method of production. In hemp, a recent study comparing different organic fertilizer regimes showed that while biomass yields were different, there was no impact on the cannabinoid ratios in the inflorescences (Bruce et al. 2022). In the current study, we wanted to evaluate the composition of epiphytic fungi found on cannabis inflorescences grown organically, which is an important quality aspect that is used to determine whether a product meets regulatory guidelines and can be sold to consumers in Canada (Bhandare 2020). In addition, we were interested to determine the occurrence of endophytic fungi, which are generally present in stem, leaf, and root tissues, in these organically grown plants. A number of these endophytes have been demonstrated to provide beneficial effects in other crop plants (Xia et al. 2015; Xia et al. 2019; de

Lamo and Takken 2020) although none have been evaluated on cannabis plants to date.

The objectives of this study, therefore, were to (1) determine the prevalence of fungi found associated with the inflorescences of organically grown cannabis plants as epiphytes (surface-colonizing fungi) and also assess the corresponding diversity of fungi found in the air in organic production facilities and (2) determine the occurrence of endophytic fungi (found inside the stem tissues) of cannabis plants grown organically as well as the populations present in soil. As a basis for comparison, the range of fungi previously reported growing as endophytes in cannabis plants in hydroponic production using cocofibre as a substrate (Punja et al. 2019), and the range of fungal species found occurring on the surface of cannabis inflorescences as epiphytes produced using nonorganic (hereafter referred to as conventional) methods (Punja 2021*a*), were referenced from previous studies.

Materials and methods

Organic production facilities

Two licensed organically certified greenhouse facilities and one licensed outdoor organic production site were sampled during 2019–2021. All three facilities were located in the Fraser Valley of BC. Cannabis plants were grown in soil or a mixture of soil and composted plant waste materials supplemented with a range of amendments approved for organic production. These amendments included a combination of fish meal, kelp, bone meal, worm castings, composts and compost teas, various types of manures, wood bark, rock dusts, wood ash, perlite and vermiculite, depending on the organic producer. Specific amounts and ratios of each constituent were not disclosed by the licensed producers as they are considered proprietary. The growing medium was placed in pots (10 L), or in raised troughs lined with polyethylene tarp, depending on the production facility. Outdoor production was in large fabric pots. Rooted plants of several cannabis strains (genotypes) were grown in these substrates according to standard production practices for greenhouse or outdoor production. After 2–4 weeks of vegetative growth, the photoperiod was adjusted to approximately 12 h of darkness to induce flowering in the greenhouse. Throughout the year, supplementary lighting was provided to greenhouse grown plants as needed for optimal growth. Outdoor plants were placed inside polyethylene hoop houses which were covered with black tarps to reduce daylength exposure during June–September as cannabis is a short day plant. Pruning and deleafing and other horticultural practices were conducted as required. Pest control products applied in the greenhouse included Rootshield® Plus WP, containing *Trichoderma harzianum* Rifai strain T-22 and *Trichoderma virens* strain G-41 (BioWorks Inc., Victor, USA), and BotaniGard® ES containing *Beauveria bassiana* strain GHA (Lam International Corporation, Butte, USA) to manage fungal pathogens and insect pests, respectively. In addition, potassium bicarbonate (Milstop), hydrogen peroxide (Zerotol), Regalia (extract from giant knotweed), and various oils were applied to the foliage of organically grown plants to reduce powdery mildew infection (Scott and Punja 2022).

Sampling schedule

To ensure standardization of results for comparative purposes, sampling for fungi present on inflorescences was conducted during weeks 5 through 8 of the flowering period. Samples were obtained from at least four different cropping cycles indoors and two cycles outdoors. Each cropping cycle was approximately 3 months in duration. For detection of endophytes, plants were sampled only at harvest time from four cropping cycles in one greenhouse facility. No outdoor samples were available. Soil samples were collected at various times during production in the greenhouse facility. The frequency of sampling and number of samples obtained are indicated below.

Inflorescence sampling

To determine the presence of epiphytic fungi on the surface of cannabis inflorescences, a swab method using sterile Q-tips as described by Punja (2021*a*) was used. Briefly, Q-tips were gently swabbed over the surface of the inflorescence tissues consisting of aggregated pistils (Fig. 1A) and immediately transferred to a Petri dish containing potato dextrose agar with 140 mg/L of streptomycin sulfate (PDA + S). The Q-tip was wiped across the surface of the agar in a zigzag pattern and discarded. A minimum of 10 inflorescences were included each time, selected at random. This procedure was conducted during September 2019–December 2021, to 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. The sampling time varied during the year depending on the availability of the maturing inflorescences in each greenhouse. In addition, approximately 20 sampling times were performed on harvested and dried cannabis inflorescences that were swabbed in a similar manner. For the outdoor location, approximately 20 sampling times were performed on inflorescences towards harvest in each of two growing seasons from one facility. The total number of Petri dishes was approximately 1000 for this study. 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. After that time, each dish was examined for the presence of fungal colonies and morphologically distinct colonies were recorded and transferred to PDA + S. After 2 weeks, they were subcultured again to ensure purity prior to identification.

Air sampling

Air sampling was conducted in the two indoor greenhouse facilities. Petri dishes containing PDA + S were placed inside the greenhouse, close to the inflorescences or adjacent to plants where they were left with lids removed for approximately 60 min (Fig. 1B). The sampling was typically conducted between 11:00 am and 2:00 pm using a total of 10–15 Petri dishes. The lids were replaced and the dishes taken to the laboratory for identification of morphologically distinct colonies

Fig. 1. Sampling methods used to assess epiphytes and endophytes in this study. (A) The swab method where a Q-tip was used to swab the surface of cannabis inflorescences and then streaked across a Petri dish containing potato dextrose agar with 140 mg/L streptomycin sulfate (PDA + S). (B) The air sampling method where Petri dishes containing PDA + S were placed within the plant canopy adjacent to cannabis inflorescences for a period of 60 min and returned to the laboratory and examined for colonies. (C, D) The whole plant assessment method for stem endophytes. Plants were obtained at harvest and after all side branches were removed, the main stem was divided into 12 cm sections starting at the bottom and progressing to the top of the plant, as shown in (D). (E) Internal stem pieces were obtained after surface-sterilization and plated onto PDA + S. (F) Endophytes, mostly *Penicillium* spp., growing out from stem pieces. (G) Soil was assessed for fungi following a dilution series and plated out onto PDA + S.

of fungal species as described above. A total number of 900 Petri dishes were included in this study.

Endophyte sampling

To assess for stem endophytes, entire plants were sampled at harvest time after inflorescences were removed. The plants selected appeared healthy with no visible symptoms of disease or pest problems (Fig. 1C). The side branches were removed and the entire main stem was divided into 12.5 cm (5 in.) segments (Fig. 1D) and these segments were transported to the laboratory in plastic bags. Root samples were also collected by excising a portion of the root system that included main and lateral roots. From each stem segment, tissue pieces measuring 0.5 cm (in diameter) were taken after the stem was sterilized by immersing in 20% bleach (Javex, containing 6.25% NaOCl) for 3 min, followed by 70% EtOH for 1 min, and rinsing thrice in sterile distilled water. Five pieces were placed on each Petri dish containing PDA + S, with four dishes per stem segment (Fig. 1E). The procedure for sterilizing the root samples was as follows: immersion in a 0.5% NaOCl solution for 30 s followed by 20 s in 70% EtOH, rinsing thrice in sterile water, blotting on sterile paper towels, after which 0.4 cm long pieces were plated out onto each of four Petri dishes. All dishes were incubated under laboratory conditions and fungal colonies identified as described below. A total of eight plants were included in this study, each divided into roughly eight segments, with 40 Petri dishes per plant (stems and roots combined). In addition to the whole plant sampling, the bottom 12.5 cm of stems from harvested plants was also sampled at time of harvest. These stem segments were sterilized, plated, and the resultant fungal colonies (Fig. 1F) were identified in the same manner as the stems sampled from the whole plant dissections. To examine for the presence of potential fungi in the pith tissues of stems, stem segments were prepared for scanning electron microscopy as described by Punja et al. (2019).

Soil sampling

Samples of growing substrate (soil mixtures) were collected from unused batches, as well as at 1 and 3 weeks post-planting, and at harvest time (8 weeks) from the root zone. Each sample weighed approximately 200 g. A subsample of 0.5 g was suspended in 10 mL of sterile distilled water and mixed using a vortex mixer for 20 s. A 1 mL suspension was transferred to 9 mL of water, shaken, and a further dilution was made in 9 mL of water. This was repeated up to a 10^{-3} dilution. Aliquots (0.5 mL) of each suspension were plated onto five replicate PDA + S plates and repeated three times for each sample. Dishes were incubated for observation of fungal colony development as described above (Fig. 1G). The remaining soil was sent to a commercial laboratory (A&L Labs. Inc., London, Canada) for analysis of total yeast and mold (TYM) levels, total aerobic bacteria, and total Gram negative bile-tolerant bacteria as per standard protocols (https://www.alcanada.com/content/solutions/cannabis-analysis?title=Microbiological%20Scan). Sample collection was repeated over two cropping cycles in one greenhouse facility. For comparison, samples of cocofibre growing substrate used in a conventional hydroponic production facility were also taken at 0, 1, 3, and 8 weeks in the production cycle to provide a comparison of the microbial populations present. The sampling was conducted in two different cropping cycles.

Identification of fungal species

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 (Punja 2021*b*). DNA was extracted from mycelium scraped from the surface of colonies on PDA + S using the QIAGEN DNeasy Plant Mini Kit. Aliquots of 1 μL containing 5–20 ng DNA were used for PCR in a 25 μL reaction volume consisting of 2.5 μL 10× buffer (containing 15 mM $MgCl_2$), 0.5 μL 10 mM dNTP, 0.25 μL Taq DNA Polymerase (QIAGEN, Venlo, The Netherlands), 0.25 μL 10 mM forward and reverse primers, as well as 20.25 μL DNase- and RNAse-free water (Invitrogen, Waltham, USA). The universal eukaryotic primers UN-UP18 S42 (5′-CGTAACAAGGTTTCCGTAGGTGAAC-3′) and UN-LO28 S576B (5′-GTTTCTTTTCCTCCGCTTATTAATATG-3′) were used (Punja 2021*b*). All PCR amplifications were performed in a MyCycler thermocycler (Bio-Rad Laboratories, Hercules, USA) with the following program: 3 min at 94 °C; 30 s at 94 °C, 30 s at 60 °C, 3 min at 72 °C (35 cycles); and 7 min at 72 °C. PCR products were separated on 1% agarose gels and bands of the expected size (ca. 700 bp) were purified with QIAquick Gel Extraction Kit and sent to Eurofins Genomics (Eurofins MWG Operon LLC 2016, Louisville, USA) for sequencing. 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%. A total number of 100 fungal colonies was analyzed. GenBank accession numbers for each of the unique fungal species recovered are presented in Supplementary Table S1.

Scanning electron microscopy

Samples of inflorescences and stem segments were prepared for scanning electron microscopy to examine for the presence of fungal spores. Pieces of bract tissues from cannabis inflorescences, measuring 0.5 × 0.5 cm and bearing pistils and trichomes, and sections made through stem tissues, 0.5 cm in length, were prepared according to the method described by Punja et al. (2019). Samples were examined at various magnifications for any spore-like structures on stigmas, trichomes, and in the pith tissue.

Comparing species diversity

The total number of different fungal species was compared relative to the source of the sample—from inflorescences, air, and soil from two indoor production facilities and from inflorescences from one outdoor production site. Since the actual numbers of colonies of each species recovered on each plate were not recorded, indices of fungal diversity could not be calculated. Instead, comparisons of the total numbers of

different genera and species in each sample were made and are presented.

Results

The air sampling Petri dishes that were placed in the organic greenhouse production facilities for a 1 h exposure duration (Fig. 1B) displayed a wide spectrum of fungal colonies growing after 5 days of incubation in the laboratory (Figs. 2A–2C). The most commonly observed fungal genera were *Penicillium*, *Alternaria*, *Cladosporium*, *Aspergillus*, *Mucor*, *Epicoccum*, and *Fusarium*. The various species found to be present in the air were identified following comparison of PCR-derived sequences in GenBank and consisted of 39 species belonging to 26 genera (Fig. 3). Swabs taken from developing inflorescences (Fig. 1A) and from select dried cannabis samples at various times similarly showed a wide diversity of fungal colonies growing on PDA + S after 5 days of incubation (Figs. 2D–2H). These included a similar range of fungal genera that were observed on the air sampling plates. A total of 29 species belonging to 16 genera were present on the inflorescence samples. In one outdoor organic facility, 11 genera and 17 species of fungi were identified (Fig. 3). The fungal species observed on inflorescences outdoors vs. indoors were very similar but since only one outdoor facility was sampled in this study, more extensive sampling of other facilities will be needed to allow a comparison to be made on the fungal populations outdoors compared to indoors. The relative abundance of fungal species, expressed as the frequency of occurrence of colonies on Petri dishes originating from indoor inflorescences, air and soil samples is shown in Supplementary Table S1. The most prevalent genera were *Penicillium*, *Aspergillus*, *Alternaria*, *Cladosporium*, and *Mucor*. Sampling of inflorescences revealed that multiple species of fungi were present at any given time, as can be seen on the swab plates (Fig. 2). The swab method of sampling recovered viable spores/mycelium of the various fungi that produced colonies on PDA + S as summarized in Fig. 3. Scanning electron microscopy showed the abundant stigmatic hairs (Figs. 4A and 4B) and the large numbers of glandular trichomes that are normally present on cannabis inflorescences (Figs. 4C and 4D). Fungal spores resembling *Penicillium* spp. could be observed on the surface of inflorescence leaves (Figs. 4E and 4F). In addition, these spores were found adhered to the surface of trichome glands (Fig. 4G) and magnified views revealed the presence of spores which resembled *Aspergillus*, *Penicillium*, and *Cladosporium* (Figs. 4H and 4I). Furthermore, resin secreted from the trichome glands was observed to have fungal spores embedded in it (Figs. 4J–4L), mostly resembling those of *Aspergillus* spp.

The analysis of samples of growing substrate for total microbial activity, including bacteria and total yeasts and molds (Table 1), showed extremely high microbial levels in organic growing substrates compared to cocofibre substrates used in hydroponic production. The highest population levels of total yeasts and molds, total aerobic bacteria, and Gram negative bile-tolerant bacteria were seen from 3 weeks of plant growth up to harvest time (8 weeks) when compared to 1 week post-planting or prior to planting. The average fungal population levels in organic soil were approximately 158 times higher than in cocofibre substrate (Table 1). Bacterial populations were up to 1360 times higher in organic substrate compared to conventional growing substrate. Soil dilution and plating revealed a limited number of genera and species to be present (11 genera and 15 species), in part because of the propensity of fast-growing colonies, such as *Mucor* and *Rhizopus* spp., which grew over the dishes and obscured the presence of many other colonies which could not be isolated and identified (Figs. 2I–2K).

The second aspect of this study was to assess the extent of fungal endophytes present in cannabis stem tissues at harvest. In this context, both plant pathogens and saprophytes were recovered from asymptomatic tissues (Fig. 5A). Several species of *Fusarium*, *Botrytis cinerea*, and *Penicillium* spp. were recovered from internal stem tissues in this study. The recovery of these fungi was consistent in stem samples from the bottom of the plant (0 cm) to the very top (75 cm) (Fig. 5). A number of other endophytic fungi, including *Acremonium alternatum*, *Lecanacillium aphanocladii*, *Metarhizium anisopliae*, and *Trichoderma harzianum*, were recovered from cannabis stem tissues in the present study. Figure 5 shows the colonies recovered of *Botrytis cinerea* (Fig. 5B), *Fusarium oxysporum* (Fig. 5C), *Aspergillus puniceus*, and *Penicillium polonicum* (Fig. 5D), *Penicillium chrysogenum* (Fig. 5E), *Chaetomium brasiliensis* (Fig. 5F), and *Penicillium olsonii* (Fig. 5G) from surface-sterilized stem sections of mature cannabis plants. *Chaetomium globosum* was also recovered sporadically from stem tissues in this study. By far, the most commonly isolated endophytes were *Penicillium* spp. Two species of *Humicola* were recovered from cannabis roots in this study. Sequences of unique and previously unreported fungi from organic cannabis facilities have been deposited in GenBank (see Supplementary Table S1).

Discussion

In a previous 2 year study which involved sampling of cannabis inflorescences within drying rooms of several licensed production facilities using the Q-tip method, 34 species of fungi belonging to 10 genera were reported to be present (Punja 2021*a*). Among them, *Penicillium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Rhizopus*, and *Botrytis* were the most common. 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) (Punja 2021*a*). The swab method of sampling recovered viable spores/mycelium of the various fungi described in this study. Their presence on cannabis inflorescences as epiphytes is likely due to their ability to adhere to the sticky surface of stigmatic tissues (Punja 2018), as well as on or around the abundant trichomes that are produced on bracts surrounding the pistils in cannabis plants (Punja and Ni 2021). Previous scanning electron microscopic studies showed that spores of *Penicillium* spp. can adhere to stigmatic tissues (Punja 2018), and the presence of *Aspergillus* spores was observed on the surface of trichome glands (Punja and Ni 2021). The scanning

Fig. 2. The colonies of a range of fungi recovered from air, inflorescences, and soil samples in this study. (A–C) Diverse fungi growing on Petri dishes exposed to the air in organic growing facilities. (A) *Alternaria*, *Aspergillus*, and *Penicillium* spp. can be seen. (B) *Alternaria*, *Epicoccum*, and *Mucor* can be seen. (C) *Aspergillus*, *Fusarium*, and *Penicillium* can be seen. (D–H) Colonies of fungi recovered from inflorescence swabs. (D) *Aspergillus*, *Fusarium*, and *Penicillium* can be seen on the dishes. (E) *Fusarium* and *Penicillium* are growing on the dishes. (F) *Mucor* and *Rhizopus* spp. can be seen on the dishes. (G) *Mucor* colonies have grown over the Petri dish. (H) *Trichoderma*, *Fusarium*, *Penicillium*, and *Aspergillus* are growing on the dishes. (I–K) Colonies of fungi recovered from soil. (I) *Trichoderma*, *Fusarium*, *Penicillium*, and *Aspergillus* colonies are growing on the dish. (J, K) *Mucor* and *Rhizopus* species are prevalent on the dishes.

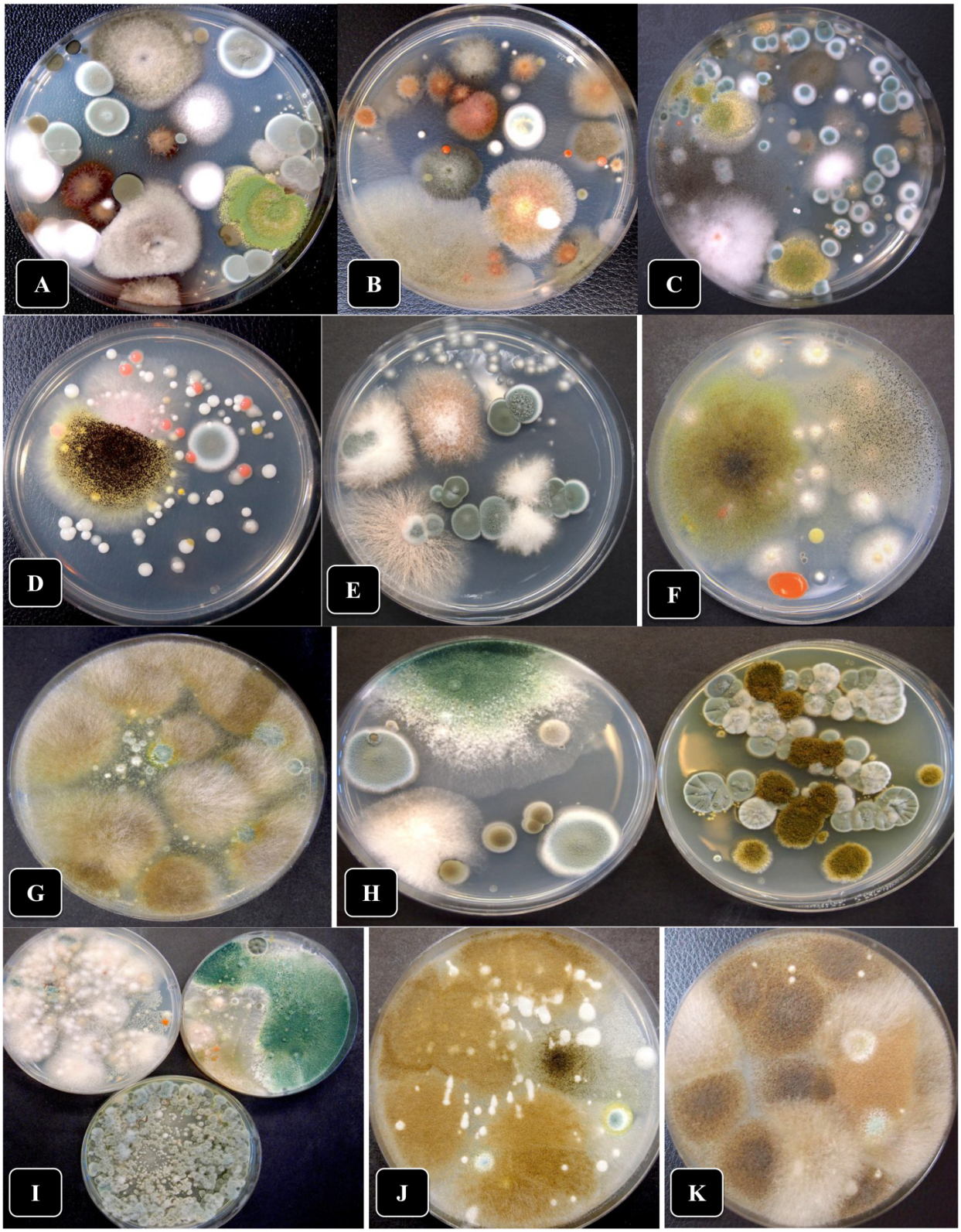


Fig. 3. Fungal species identified from comparisons of PCR-derived sequences of the ITS1-5.8S-ITS2 region of ribosomal deoxyribonucleic acid. Species recovered from air, soil, and inflorescences from an indoor cannabis organic facility and from inflorescences from an outdoor facility are indicated. Similar colored symbols indicate that particular species was present at each sampling site.

images from the present study showed that spores stuck to trichome glands resembled those of *Botrytis*, *Aspergillus*, *Cladosporium*, as well as other fungi that may be present in inflorescences while adhered to the sticky surfaces of stigmas and trichome glands. The swab method of sampling likely recovered a portion of these fungi that were present as viable epiphytes. There is no previously published information to demonstrate if the resinous content of cannabis trichome glands is inhibitory to fungal spore germination but the recovery of viable colonies suggests it may not be. The effects of resinous trichome secretions on fungal spore germination is worthy of further study.

Sampling of inflorescences revealed that multiple species of fungi were present at any given time, many of which are previously unreported from cannabis inflorescences. In particular, *Conidiobolus coronatus*, *Nigrospora oryzae*, *Epicoccum nigrum*, *Mucor circinelloides*, and *Mucor racemosus* were recovered in this study. None of these species were previously observed

Fig. 4. Scanning electron microscopic images of cannabis inflorescences to show the stigmas and trichomes that are present as well as spores of various fungi that adhere to these tissues. (A) A cluster of stigmas (arrow). (B) A close-up of a young stigma showing the prolific production of stigmatic hairs (arrow). (C) Glandular trichomes (arrow) produced in abundance on bract tissues surrounding the pistils. (D) A close-up view of the glandular trichomes, many of which are stalked (arrow). (E, F) Scanning electron microscopic images of the surface of inflorescence bracts showing the presence of chains of spores of *Penicillium*. (G–I) Images of spores stuck to the surface of glandular heads. (G) *Aspergillus* spores (arrow) attached to the surface of a trichome head. (H) Close-up of chain of *Aspergillus* spores. (I) A spore of *Botrytis* stuck on a trichome head. (J–L) Spores of various fungi embedded in the resinous material secreted from trichome heads. (J) Spores of *Aspergillus* embedded in the resinous material (arrow). (K, L) Spores of unidentified fungi in the resin.

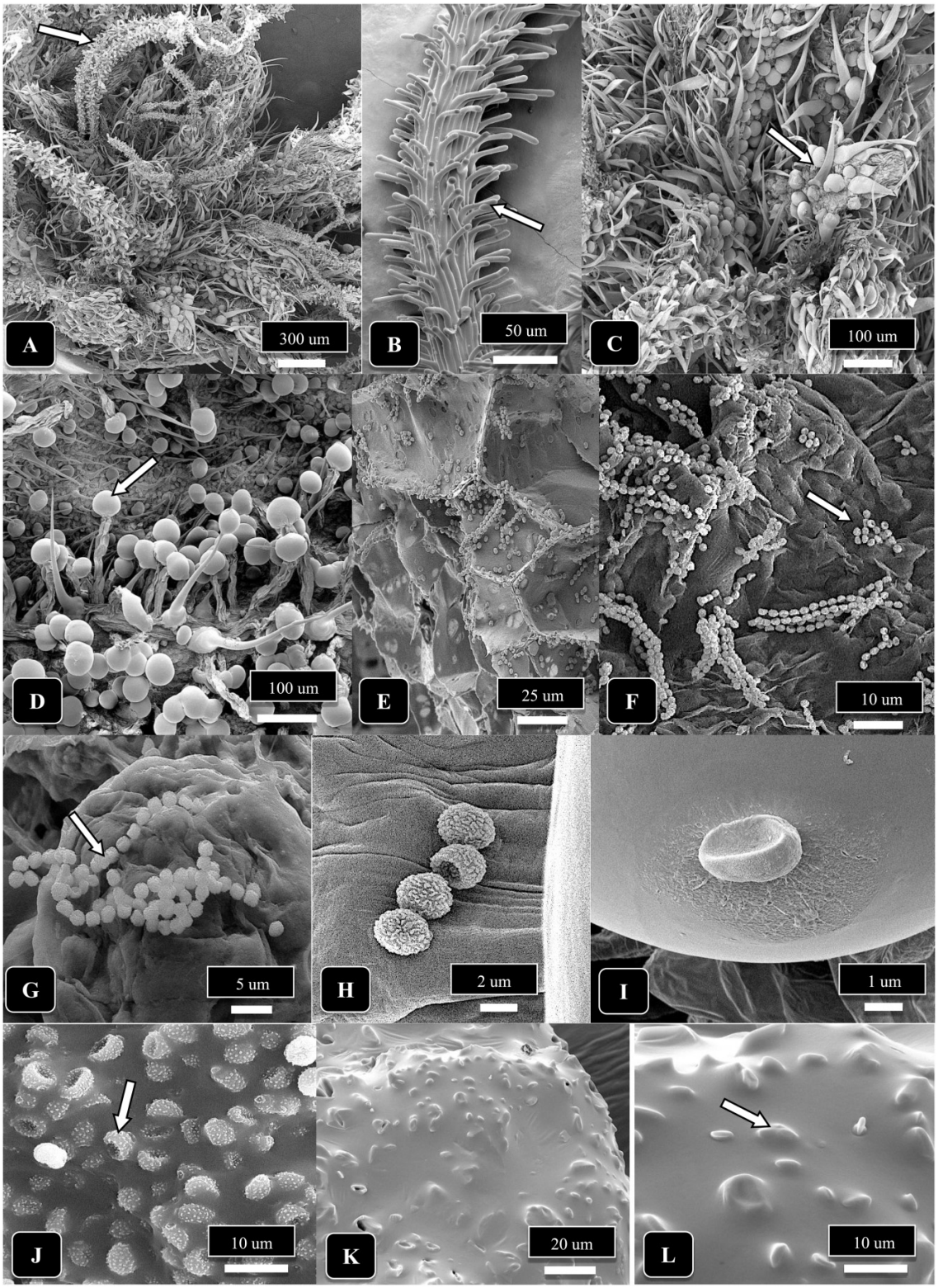


Table 1. Microbial populations in organic soil and cocofibre substrate used for cannabis production sampled at various time intervals during production.

	Microbial populations (cfu/g × 10^6)					
	Total yeast and mold		Total aerobic bacteria		Bile-tolerant Gram negative bacteria	
Sample analyzed[a]	Range	Mean	Range	Mean	Range	Mean
Organic soil						
Unused	0.45–2.2	0.87	14–44	30	0.45–2.9	1.36
1 week post-planting	5.6–12.5	7.65	400–760	460	4.4–13.1	9.4
3 weeks post-planting	18–29	23	365–1145	780	15.8–32.4	19.8
At harvest (8 weeks)	6.0–11.9	8.4	820–1255	1121	3.5–10.1	8.1
Cocofibre substrate						
Unused (dry)	0.001–0.06	0.0055	0.12–0.3	0.07	0.001–0.09	0.001
Unused (wet)	0.09–2.1	0.45	2.1–15.8	8.1	0.01–0.89	0.036
1 week post-planting	0.25–2.1	0.78	7.1–15.7	11.4	0.11–1.9	0.47
3 weeks post-planting	0.9–3.3	1.26	19.2–31.1	22.4	0.4–1.3	0.72
At harvest (8 weeks)	0.23–1.54	0.69	12–30	18.0	0.5–3.6	1.9

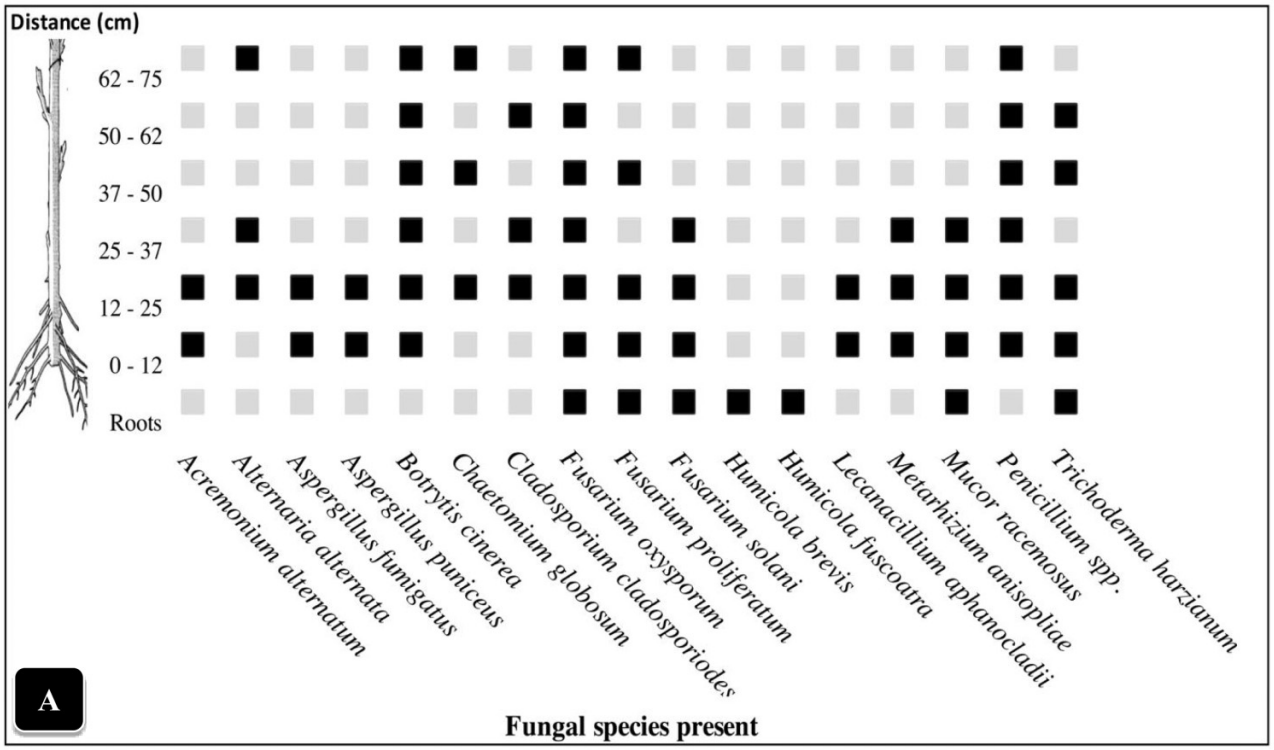
[a]Testing of all soil and cocofibre samples was conducted by a commercial laboratory (A&L Labs). The ranges and means shown are from three samples taken during different cropping cycles.

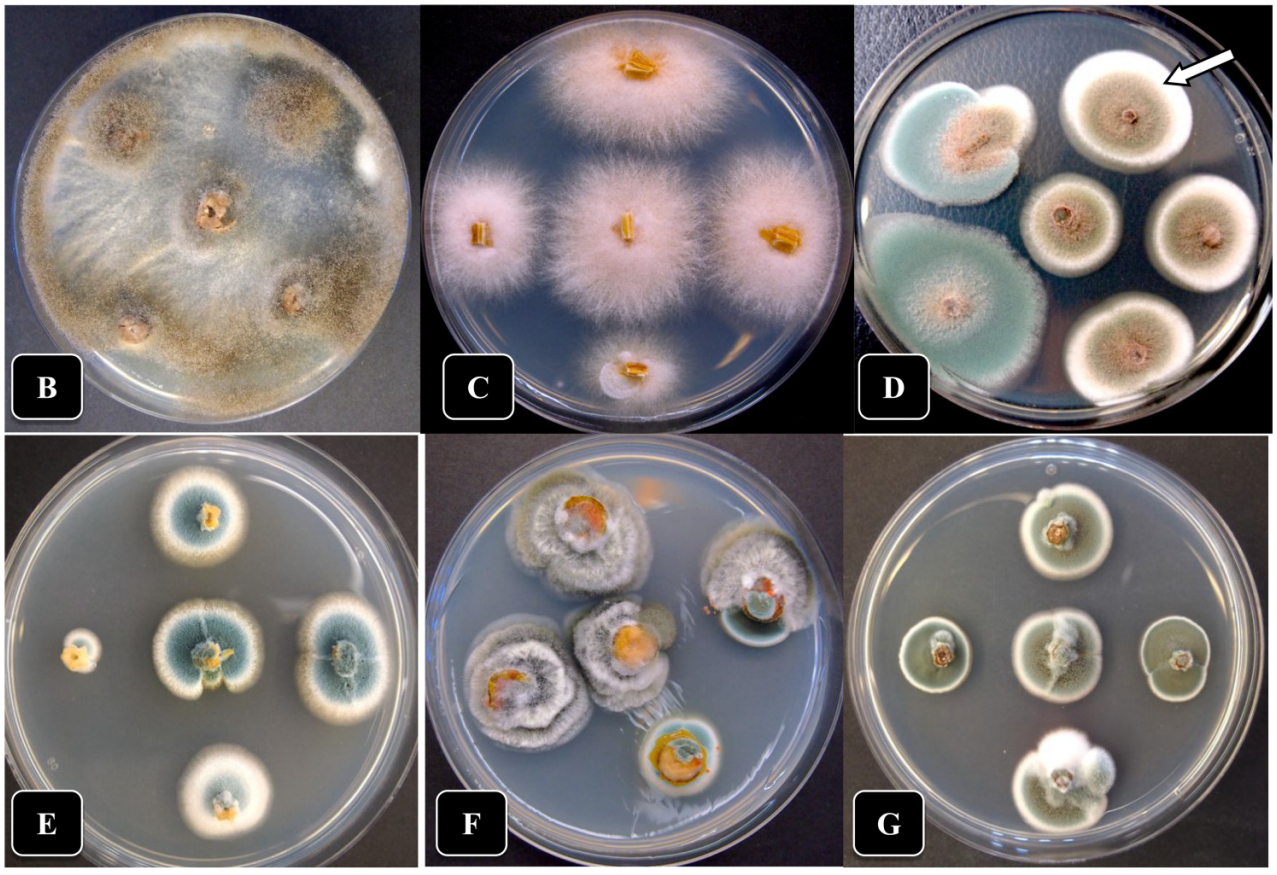
on samples which originated from conventional growing facilities (Punja 2021*a*). These fungi are all saprophytes, found on decomposing plant materials, and mostly present in soil. They are prolific spore producers and were also found to be present in the air in this study. The high diversity of fungal species present in organic production greenhouses, both in the air and on inflorescences, is assumed to have originated from the organic soil, which contained a significant high overall microbial activity (comprising both fungi and bacteria). Organic soils have been consistently reported to contain higher levels of microbial activity and a greater diversity of species compared to soils originating from conventional cropping systems (Lupatini et al. 2017; Gazdag et al. 2019; Xia et al. 2019; Fadiji et al. 2020; Visioli et al. 2020Xia et al. 2015). This diversity has been correlated with a greater ability of organic soils, or soils amended with organic constituents, to suppress the development of plant pathogens due to the presence of competing microorganisms, particularly bacteria, as well as fungal endophytes (Bonanomi et al. 2010; Li et al. 2019; De Corato 2020; Tao et al. 2020; Vida et al. 2019). This aspect deserves further study with regard to the potential of organic soils to suppress root-infecting pathogens affecting cannabis plants. Several pathogens, such as *Fusarium* and *Pythium* species, cause a large number of diseases on hydroponically grown cannabis in greenhouses (Punja 2021*b*; Punja et al. 2022; Scott and Punja 2022), where disease suppression is unlikely to be present due to the absence of competing microflora. Therefore, organic soils may offer an advantage in terms of providing greater disease suppression but further research is required to demonstrate this.

Of interest pertaining to the range of fungi present in organic cannabis production facilities was the identification of several opportunistic human pathogens. For example, *Conidiobolus coronatus* and *Nigrospora oryzae* were recovered from air, soil, and inflorescences in this study (Fig. 3). The former can cause rhinofacial conidiobolomycosis, especially in tropical countries, and was shown to infect healthy farm workers between the ages of 20 and 60 (Chowdhary et al. 2010; Deak et al. 2018). Infection by *Conidiobolus coronatus* likely occurs due to inhalation of fungal spores which imbed themselves into the nasal mucosa; subsequently, they penetrate into the subcutaneous area of the face as well as the nasal cavity and sinuses, causing facial deformation with extensive nasal blockage and bleeding. *Nigrospora oryzae* is reported to be a plant pathogen, causing diseases on rice, Indian mustard, bamboo, and hemp plants (Sharma et al. 2013; Hao et al. 2020; Liu et al. 2021; Balthazar et al. 2022) as well as being an opportunistic human pathogen, causing pulmonary and skin infections (Vanam et al. 2020). It is also reported to be an endophyte (Wang et al. 2017). Other fungi detected in organic production facilities and not previously found in conventional cannabis facilities include *Mucor circinelloides* and *Mucor racemosus*. These fungi grow rapidly, are prolific spore producers, and are commonly found on decomposing organic matter in soil, and frequently are components of the mycoflora in air. They are also reported to cause post-harvest diseases in plants (Kwon and Hong 2005; Park et al. 2014; Saito et al. 2016). Both species are considered opportunistic human pathogens, causing mucormycosis, which can result in potentially fatal infections in immunocompromised patients and cause problematic infections in young children (Vellanki et al. 2018). A recent outbreak of mucormycosis in India and other countries was especially prevalent in recovering Covid-19 patients (Borkar 2021; Hoenigl et al. 2022). These fungi have also been reported to cause allergies and inflammation of sinuses—their allergenicity has made them a common inclusion in routine allergen medical testing. Other fungi that are potentially associated with allergies that were present in the air in this study, and also reported to be present in conventional cannabis production facilities, included species of *Alternaria*, *Cladosporium*, *Fusarium*, and *Aspergillus* (Punja et al. 2019; Ma et al. 2021).

Fungi unique to organic production facilities present in the air in this study were *Acremonium alternatum* (a hyperparasite

Fig. 5. (A) The various endophytic fungal species recovered from cannabis stem tissues at various distances away from the roots. The black squares indicate the presence of each species at the specific distances indicated on the left. (B–G) Colonies of various endophytic fungi recovered from cannabis stem tissues growing on potato dextrose agar + 140 mg streptomycin sulfate. Photos were taken after 7 days of incubation at ambient room temperature. (B) *Botrytis cinerea*. (C) *Fusarium oxysporum*. (D) *Aspergillus puniceus* (arrow) and *Penicillium polonicum* (blue colonies). (E) *Penicillium chrysogenum*. (F) *Chaetomium brasiliense*. (G) *Penicillium olsonii*.





of powdery mildew and an endophyte) (Malathrakis 1985), *Bjerkandera adusta* (a basidiomycete causing white rot and found on dead trees) (Wang et al. 2021), *Cercospora canescens* (causing cercospora leaf spot on mungbean) (Mew et al. 1975), *Humicola brevis* (a thermophilic soil inhabiting ascomycete common in composts and an endophyte) (Christensen et al. 1962), *Hydnopolyporus (Polyporus) fimbriatus* (a common wood rotting basidiomycete) (Fidalgo 1963), *Mortierella hyalina* (a soil inhabiting fungus and an endophyte) (Ozimek and Hanaka 2021), *Paraphaeosphaeria michotii* (an ascomycete causing leaf spot on grasses) (Shoemaker and Eriksson 1967), *Stemphylium versicarium* (a pathogen of alfalfa and asparagus) (Foster et al. 2019), and *Scedosporium aurantiacum* (a soil-inhabiting ascomycete) (Kaur et al. 2019). The sources of origin of these fungi may be from fields and crops (including blueberry, tomato, and pasture hay) growing in the vicinity of the cannabis greenhouses, in addition to the organic growing substrate. Sequences of unique and previously unreported fungi from organic cannabis facilities have been deposited in GenBank (see Supplementary Table S1). It should be noted that these identifications were made solely from sequences of the ITS region, and while a sequence similarity of 99% or higher was used, additional gene sequences in addition to the ITS could have been useful to support the identities of these species.

The detection of *Scedosporium aurantiacum* in an 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 (Cortez et al. 2008; Kaur et al. 2019; Mizusawa et al. 2021). This ascomycete fungus has been recovered from soil, sewage, and cattle, and poultry manures (Cortez et al. 2008). Consistently, *Scedosporium* is considered the second most frequent filamentous fungal genus (after *Aspergillus*) to colonize the lungs of patients with cystic fibrosis, and can cause invasive infections in transplant recipients and patients with haematological diseases, resulting in a progressive and severe deterioration of lung function of these individuals over time (Kaur et al. 2019). While *Aspergillus* species are currently recognized to be of concern by Health Canada and the United States Department of Agriculture if present on cannabis products, the occurrence of *Scedosporium* warrants further consideration, although it was not specifically found on cannabis inflorescences but was present in air samples in the growing environment.

During organic crop production, a range of naturally occurring inputs from plant, animal, and mineral sources may be used. The specific inputs allowed can vary according to crop and geographic region. Two regulatory agencies, the USDA-National Organic Program and the CFIA-Canada Organic Regime, regulate organic food production in the USA and Canada, respectively. Since cannabis is not considered a food product, however, guidelines do not currently exist at the national level to guide organic cannabis producers. In the present study, the specific components and ratios used to prepare the growing substrate within each facility were not disclosed, but the inclusion of various forms of manures, fish meal, wood chips, and bark mulch, was confirmed at both production sites. A range of similar inputs were evaluated for organic hemp production (Bruce et al. 2022). These substrates contain high levels of carbon, nitrogen, and other nutrients that can cause a proliferation of microbes over a short time period, many of which can subsequently become airborne as bioaerosols and potentially contaminate cannabis inflorescences. In mushroom cultivation facilities that utilize varying sources of composts as a substrate, the occurrence of high numbers of airborne fungal spores has raised concerns over the potential for inhalation by workers and triggering asthma and other respiratory problems (Tanaka et al. 2001; Ampere et al. 2012). Similar findings were reported in compost manufacturing facilities, where human exposure to airborne spores was reported to be of potential concern (Forestier et al. 2008; Wery 2014). In these environments, aerosols contained a range of fungi, including species of *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Mucor*, *Rhizopus*, and *Conidiobolus*, among others (Wery 2014). Interestingly, all of these fungi were present in the organic cannabis production facilities sampled in this study. A number of actinomycetes were also present in bioaerosols originating from composts (Wery 2014); these were not assessed in the present study.

Final TYM levels present on cannabis flowers post-harvest, which are reported as colony forming units (cfu) per gram, is an important regulatory requirement in Canada, the USA, and other countries around the world to ensure that potentially harmful microbes are either absent or present at permissible levels in the product at point of sale to consumers. Commercial testing laboratories carry out the required analyses and TYM numbers exceeding a specified limit (ranging from 1000–10 000 up to 50 000–100 000 cfu/g depending on the jurisdiction) can cause a product to be rejected. In Canada, the current limit is set at 50 000 cfu/g (Health Canada 2019). Other than the requirement for reporting the species of toxigenic *Aspergillus* and bacteria such as *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus*, the identification of other fungal species that could potentially cause harm to humans is not specified in current regulations. In the present study and in previous work (Punja 2021*a*), identification of fungal species using molecular approaches was considered an important component to estimate the prevalence and types of fungi found on cannabis inflorescences during production. Our data indicate that there is a higher diversity of fungal species, as well as higher microbial load in organic growing substrates and indoor environments, which could present an additional challenge for organic producers of cannabis. It is conceivable that these growing conditions can result in higher rates of contamination of inflorescences in the final product; however, we have no data to support this at the present time. What is probable is that the high diversity and presence of some potentially harmful fungal species in the air could be a concern for workers in organic indoor facilities, who may be exposed to inhalation of fungal spores. For example, during indoor and outdoor cannabis harvesting and trimming operations, high levels of airborne bacteria, actinomycetes, and fungi were reported to be encountered by workers (Martyny et al. 2013: Green et al. 2018); the most prevalent fungal genera were *Penicillium*, *Cladosporium*, and

Botrytis. Air sampling in organic cannabis production facilities, as conducted in this study, has the advantage of identifying airborne populations of fungi that could potentially contaminate cannabis inflorescences.

A range of endophytic fungi were recovered from cannabis stem tissues in this study, some of which have been recovered previously from stems of plants grown in hydroponic production (Punja et al. 2019). These include *Fusarium* and *Penicillium* spp. These endophytes have been shown to be problematic in tissue culture studies of cannabis, as despite rigorous surface-sterilization attempts, they can still be present as internal contaminants (Holmes et al. 2021). The potential roles of the *Penicillium* species within cannabis tissues remain unexplored. The recovery of *Fusarium* spp. as endophytes in cannabis tissues was reported previously (Punja et al. 2019). Similarly, *Aspergillus* species and *Chaetomium globosum* have been reported as endophytes in cannabis stem and leaf tissues (Punja et al. 2019). A number of potentially beneficial endophytic fungi that are reported to promote growth in other plant species, including *Acremonium alternatum*, *Lecanacillium aphanocladii*, *Metarhizium anisopliae*, and *Trichoderma harzianum*, were also recovered from cannabis stem tissues in the present study. Species of *Humicola* were also recovered from roots in this study. Members of this genus are commonly found in soil, composts, and decaying plant materials (Wang et al. 2019). Some species are endophytic and have potential as biological control agents of plant diseases (Wang et al. 2019). Infection of tomato roots by *Humicola fuscoatra* was not reported to cause any visible disease symptoms (Menzies et al. 1998). A few species may cause allergies in humans (Wang et al. 2019). On outdoor grown hemp plants, *Alternaria* and *Cochliobolus* were the most prevalent fungal genera recovered (Scott et al. 2018). Further studies are needed to determine the possible benefits to cannabis growth by various endophytic fungi harboured in root and stem tissues.

The nature of plant–endophyte interactions ranges from mutualism to pathogenicity, depending on many abiotic and biotic factors, including the genotypes of plants and microbes, environmental conditions, nutrient availability, and the dynamic network of interactions within the plant microbiome (Hardoim et al. 2015; Mengistu 2020; Thoms et al. 2021). With regard to the potential for endophytes to reduce plant diseases, Busby et al. (2016) stated "Fungal endophyte effects on plant disease severity are context-dependent. The complexity within plant microbiomes presents a significant challenge to disentangling the biotic environmental factors affecting plant disease severity". Therefore, carefully executed studies are needed to elucidate the roles of endophytic microbes within cannabis tissues since their potential benefits as endophytes can only be established through experimentation. Their recovery from cannabis plants alone cannot be assumed to provide benefits until such data are available.

An interesting observation was the recovery of *Botrytis cinerea* from healthy stem tissues in the present study, where it presumably resided as an endophyte without causing disease symptoms. These cryptic infections have been described for *Botrytis cinerea* on a range of host plants, where there were no obvious deleterious effects on the growth of the plant despite the presence of the pathogen (van Kan et al. 2014; Shaw et al. 2016). In some cases, quiescent presence of *Botrytis cinerea* inside plant tissues could occur for weeks (van Kan et al. 2014). The prevalence of these cryptic infections appeared to be greater when plants were grown under high light intensity, which is commonly encountered in cannabis production. The isolates of *Botrytis cinerea* recovered as endophytes were shown to be pathogenic (Shaw et al. 2016). Sporulation and disease symptoms did develop on symptomless plants, however, when infected tissues were stressed, or became mature, or senescent (van Kan et al. 2014; Shaw et al. 2016). The cannabis plants from which *Botrytis cinerea* was isolated were not showing any visual symptoms and tissues were collected at harvest. The endophytic growth of *Botrytis cinerea* in cannabis stem tissues as a latent pathogen and its ability to cause disease at some point later during plant development has not been determined and the importance of this cryptic phase as a source of inoculum is unknown. Generally, *Botrytis cinerea* primarily causes inflorescence rot on cannabis plants although stem cankers are not uncommon (Punja and Ni 2021). The ability of *Fusarium*, *Botrytis*, and *Penicillium* spp., which were recovered from the penultimate 62–75 cm segments of the stems of mature cannabis plants in this study, to be vertically transmitted into the floral tissues (at an approximate height of 75–87 cm) is unknown. All of these pathogens are reported to cause bud rots under warm and humid conditions (Punja 2018; Punja and Ni 2021). It is conceivable that internal stem-inhabiting inoculum is capable of invading the inflorescence tissues, leading to the development of bud rot symptoms, but this requires further study.

In conclusion, the results from this study indicate 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. 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 (Punja et al. 2019). 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. The greater microbial diversity and presence of endophytes in stem tissues in organically grown cannabis requires additional studies to determine the potential benefits they may provide in protecting against plant pathogens, especially those infecting the roots. Conversely, the higher fungal spore populations in organic greenhouse facilities could lead to a higher concentration of TYM present in inflorescences that could cause products to fail to meet quality assurance criteria. These challenges need to be considered against the conventional hydroponic cultivation of cannabis, which generally has lower airborne spore concentrations but on which a larger number of plant pathogens have been described (Punja 2021*c*; Scott and Punja 2022). The insights gained from air sampling in cannabis production facilities in this study suggest that this should become a common practice for quality assurance.

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Supplementary material

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References

Ampere, A., Delhaes, L., Soots, J., Bart, F., and Wallaert, B. 2012. Hypersensitivity pneumonitis induced by shiitake mushroom spores. Med. Mycol. **50**(6): 654–657. doi:10.3109/13693786.2012.658091. PMID: 22329454.

Balthazar, C., Novinscak, A., Cantin, G., Joly, D.L., and Filion, M. 2022. Biocontrol activity of *Bacillus* spp. and *Pseudomonas* spp. against *Botrytis cinerea* and other cannabis fungal pathogens. Phytopathology, **112**(3): 549–560. doi:10.1094/PHYTO-03-21-0128-R. PMID: 34293909.

Bhandare, S. 2020. The microbiological testing regulations for cannabis products in Canada: Are they enough from food safety and public health point of view? J. Food Microbiol. Saf. Hyg. **5**: 1. doi:10.1234/4.2020.5672.

Bonanomi, G., Antignani, V., Capodilupo, M., and Scala, F. 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biol. Biochem. **42**: 136–144. doi:10.1016/j.soilbio.2009.10.012.

Borkar, S.G. 2021. Mucormycosis: a surge in Mucorales fungal infection in post-Covid patients in Indian states and insight into known and unknown factors. Int. J. Glob. Health, **1**(3): 26–60. doi:10.14302/issn.2693-1176.ijgh-21-3907.

Bruce, D., Connelly, G., and Ellison, S. 2022. Different fertility approaches in organic hemp (*Cannabis sativa* L.) production alter floral biomass yield but not CBD:THC ratio. Sustainability, **14**(10): 6222. doi:10.3390/su14106222.

Busby, P. E., Ridout, M., and Newcombe, G. 2016. Fungal endophytes: modifiers of plant disease. Plant Mol. Biol. **90**(6): 645–655. doi:10.1007/s11103-015-0412-0. PMID: 26646287.

Chowdhary, A., Randhawa, H.S., Khan, Z.U., Ahmad, S., Khanna, G., Gupta, R., and Roy, P. 2010. Rhinoentomophthoromycosis due to *Conidiobolus coronatus*. A case report and an overview of the disease in India. Med. Mycol. **48**: 870–879. doi:10.3109/13693786.2010.486010.

Christensen, M., Whittingham, W.F., and Novak, R.O. 1962. The soil microfungi of wet-mesic forests in southern Wisconsin. Mycologia, **54**(4): 374–388. doi:10.1080/00275514.1962.12025012.

Cortez, K.J., Roilides, E., Quiroz-Telles, F., Meletiadis, J., Antachopoulos, C., Knudsen, T., et al. 2008. Infections caused by *Scedosporium* spp. Clin. Microbiol. Rev. **21**(1): 157–197. doi:10.1128/CMR.00039-07. PMID: 18202441.

De Corato, U. 2020. Soil microbiota manipulation and its role in suppressing soil-borne plant pathogens in organic farming systems under the light of microbiome-assisted strategies. Chem. Biol. Technol. Agric. **7**: 17. doi:10.1186/s40538-020-00183-7.

De Lamo, F.J., and Takken, F.L.W. 2020. Biocontrol by *Fusarium oxysporum* using endophyte-mediated resistance. Front. Plant Sci. **11**: 37. doi:10.3389/fpls.2020.00037.

Deak, L., Mudalagiriyappa, S., Ballin, A., Saxton, D., and Chakrabarti, A. 2018. A rhinofacial *Conidiobolus coronatus* fungal infection presenting as an intranasal tumour. Sultan Qaboos Univ. Med. J. **18**(4): 549–552. doi:10.18295/squmj.2018.18.04.022.

Fadiji, A.E., Ayangbenro, A.S., and Babalola, O.O. 2020. Organic farming enhances the diversity and community structure of endophytic archea and fungi in maize plants: a shotgun approach. J. Soil Sci. Plant Nutr. **20**: 2587–2599. doi:10.1007/s42729-020-00324-9.

Fidalgo, O. 1963. Studies on the type species of *Hydnopolyporus*. Mycologia, **55**(6): 713–727. doi:10.2307/3756475.

Forestier, D., Thoumelin, P., Efremenko, B., and Arfi, C. 2008. Occupational bioaerosol exposure in composting plants. Waste Manag. Environ. **109**: 911–917. doi:10.2495/WM080921.

Foster, J.M., Tayviah, C.S., Stricker, S.M., Gossen, B.D., and McDonald, M.R. 2019. Susceptibility to *Stemphylium vesicarium* of asparagus, onion, pear, and rye in Canada. Can. J. Plant Pathol. **41**(2): 228–241. doi:10.1080/07060661.2019.1574901.

Gazdag, O., Kovacs, R., Paradi, I., Fuzy, A., Kodobocz, L., Mucsi, M., et al. 2019. Density and diversity of microbial symbionts under organic and conventional agricultural management. Microbes Environ. **34**(3): 234–243. doi:10.1264/jsme2.ME18138. PMID: 31189767.

Green, B.J., Couch, J.R., Lemons, A.R., Burton, N.C., Victory, K.R., Nayak, A.P., and Beezhold, D.H. 2018. Microbial hazards during harvesting and processing at an outdoor United States cannabis farm. J. Occup. Environ. Hyg. **15**(5): 430–440. doi:10.1080/15459624.2018.1432863.

Hao, Y., Aluthmuhandiram, J.V.S., Chethana, K.W.T., Manawasinghe, I.S., Li, X., Liu, M., and Zhang, W. 2020. *Nigrospora* species associated with various hosts from Shandong Peninsula, China. Mycobiology, **48**(3): 169–183. doi:10.1080/12298093.2020.1761747.

Hardoim, P.R., van Overbeek, L.S., Berg, G., Pirttila, A.M., Compant, S., Campisano, A., and Sessitsch, A. 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol. Mol. Biol. Rev. **79**(3). doi:10.1128/MMBR.00050-14. PMID: 26136581.

Health Canada. 2019. Guidance document: good production practices guide for cannabis: testing for contaminants: 5.3.1.2. Available from https://www.canada.ca/en/health-canada/services/cannabis-regulations-licensed-producers/good-production-practices-guide/guidance-document.html#a5.1 [accessed 6 March 2021].

Hoenigl, M.D., Seidel, D., Carvalho, A., Rudramurthy, S.M., Arastehfar, A., Gangneux, J-P., et al. 2022. The emergence of COVID-19 associated mucormycosis: a review of cases from 18 countries. Lancet Microbe, **3**(7): e543–e552. doi:10.1016/S2666-5247(21)00237-8. PMID: 35098179.

Holmes, J.E., Lung, S., Collyer, D., and Punja, Z.K. 2021. Variables affecting shoot growth and plantlet recovery in tissue cultures of drug-type *Cannabis sativa* L. Front. Plant Sci. **12**: 732344. doi:10.3389/fpls.2021.732344. PMID: 34621286.

Kaur, J., Kautto, L., Penesyan, A., Meyer, W., Elbourne, L.D.H., Paulsen, I.T., and Nevalainen, H. 2019. Interactions of an emerging fungal pathogen *Scedosporium aurantiacum* with human lung epithelial cells. Sci. Rep. **9**: 5035. doi:10.1038/s41598-019-41435-3.

Kwon., J.H., and Hong, S.B. 2005. Soft rot of tomato caused by *Mucor racemosus* in Korea. Mycobiology, **33**(4): 240–242. doi:10.4489/MYCO.2005.33.4.240. PMID: 24049508.

Li, H., Cai, X., Gong, J., Xu, T., Ding, G.C., and Li, J. 2019. Long-term organic farming manipulated rhizospheric microbiome and *Bacillus* antagonism against pepper blight (*Phytophthora capsici*). Front. Microbiol.doi:10.3389/fmicb.2019.00342.

Liu, L., Zhao, K., Zhao, Y., Zhang, Y., Fu, Q., and Shiwen, H. 2021. *Nigrospora oryzae* causing panicle branch rot disease on *Oryza sativa* (rice). Plant Dis. **105**(9): 2724. doi:10.1094/PDIS-11-20-2423-PDN.

Lupatini, M., Korthals, G.W., de Hollander, M., Janssens, T.K.S., and Kuramae, E.E. 2017. Soil microbiome is more heterogeneous in organic than in conventional farming system. Front. Microbiol. **7**: 2064. doi:10.3389/fmicb.2016.02064. PMID: 28101080.

Ma, X., Hu, J., Yu, C., Wang, C., Gu, Y., Cao, S., et al. 2021. Assessment of the pulmonary adaptive immune response to *Cladosporium cladosporioides* infection using an experimental mouse model. Sci. Rep. **11**: 909. doi:10.1038/s41598-020-79642-y.

Malathrakis, N.E. 1985. The fungus *Acremonium alternatum* Line: Fr., a hyperparasite of the cucurbits powdery mildew pathogen *Sphaerotheca fuliginea*. J. Plant Dis. Prot. **92**(5): 509–515. http://www.jstor.org/stable/43383066.

Martyny, J.W., Serrano, K.A., Schaeffer, J.W., and Van Dyke, M.V. 2013. Potential exposures associated with indoor marijuana growing operations. J. Occup. Environ. Hyg. **10**(11): 622–639. doi:10.1080/15459624.2013.831986. PMID: 24116667.

Mengistu, A.A. 2020. Endophytes: colonization, behaviour, and their role in defense mechanism. Int. J. Microbiol. 6827219. doi:10.1155/2020/6927219.

Menzies, J.G., Ehret, D.L., Koch, C., and Bogdanoff, C. 1998. *Humicola fuscoatra* infects tomato roots, but is not pathogenic. Eur. J. Plant Pathol. **104**: 767–775. doi:10.1023/A:1008680018677.

Mew, I.P.C., Wang, T.C., and Mew, T.W. 1975. Inoculum production and evaluation of mungbean varieties for resistance to *Cercospora canescens*. Plant Dis. Rep. **59**(5): 397–401.

Mizusawa, M., Totte, n M., and Zhang, S.X. 2021. A case of *Scedosporium aurantiacum* infection in the United States. Mycopathologia, **186**: 127–130. doi:10.1007/s11046-020-00498-x. PMID: 33392858.

Ozimek, E., and Hanaka, A. 2021. *Mortierella* species as the plant growth-promoting fungi present in agricultural soils. Agriculture, **11**(1): 7. doi:10.3390 /agriculture11010007.

Park, J.H., Cho, S.E., Lee, S.H., Lee, C.K., and Shin, H.D. 2014. First report of postharvest rot of chestnuts caused by *Mucor racemosus* f. *sphaerosporus* in Korea. Plant Dis. **98**(12): 1742–1743. doi:10.1094/PDIS-05-14-0547-PDN. PMID: 30703917.

Punja, Z.K. 2018. Flower and foliage-infecting pathogens of marijuana (*Cannabis sativa* L.) plants. Can. J. Plant Pathol. **40**: 514–527. doi:10.1080/07060661.2018.1535467.

Punja, Z.K. 2021a. The diverse mycoflora present on dried cannabis (*Cannabis sativa* L., marijuana) inflorescences in commercial production. Can. J. Plant Pathol. **43**(1): 88–100. doi:10.1080/07060661.2020.1758959.

Punja, Z.K. 2021b. Epidemiology of *Fusarium oxysporum* causing root and crown rot of cannabis (*Cannabis sativa* L., marijuana) plants in commercial greenhouse production. Can. J. Plant Pathol. **43**(2): 216–235. doi:10.1080/07060661.2020.1788165.

Punja, Z.K. 2021c. Emerging diseases of *Cannabis sativa* and sustainable management. Pest Manag. Sci. **2021**. doi:10.1002/ps.6307.

Punja, Z.K., and Ni, L. 2021. The bud rot pathogens infecting cannabis (*Cannabis sativa* L., marijuana) inflorescences: symptomology, species identification, pathogenicity and biological control. Can. J. Plant Pathol. **43**(6): 827–854. doi:10.1080/07060661.2021.1936650.

Punja, Z.K., Collyer, D., Scott, C., Lung, S., Holmes, J., and Sutton, D. 2019. Pathogens and molds affecting production and quality of *Cannabis sativa* L. Front. Plant Sci. **10**: 1120. doi:10.3389/fpls.2019.01120. PMID: 31681341.

Punja, Z.K., Scott, C., and Lung, S. 2022. Several *Pythium* species cause crown and root rot on cannabis (*Cannabis sativa* L., marijuana) plants grown under commercial greenhouse conditions. Can. J. Plant Pathol. **44**(1): 66–81. doi:10.1080/07060661.2021.1954695.

Reganold, J.P., and Wachter, J.M. 2016. Organic agriculture in the twenty-first century. Nat. Plants, **2**: 15221. doi:10.1038/NPLANTS.2015.221. PMID: 27249193.

Saito, S., Michailides, T.J., and Xiao, C.L. 2016. Mucor rot – an emerging postharvest disease of mandarin fruit caused by *Mucor piriformis* and other *Mucor* spp. in California. Plant Dis. **100**: 1054–1063. doi:10.1094/PDIS-10-15-1173-RE. PMID: 30682278.

Scott, C., and Punja, Z.K. 2022. Management of diseases on cannabis in controlled environment production. *In* Handbook of cannabis production in controlled environments. *Edited by* Y. Zheng. CRC Press.

Scott, M., Rani, M., Samsatly, J., Charron, J.-B., and Jabaji, S. 2018. Endophytes of industrial hemp (*Cannabis sativa* L.) cultivars: identification of cultural bacteria and fungi in leaves, petioles, and seeds. Can. J. Microbiol. **64**(10): 664–680. doi:10.1139/cjm-2018-0108. PMID: 29911410.

Sharma, P., Meena, PD., and Chauhan, J.S. 2013. First report of *Nigrospora oryzae* (Berk. & Broome) Petch causing stem blight on *Brassica juncea* in India. J. Phytopathol. **161**(6): 440–441. doi:10.1111/jph.12081.

Shaw, M.W., Emmanuel, C.J., Emilda, D., Terhem, R.B., Shafia, A., Tsamaidi, D., and van Kan, J.A.L. 2016. Analysis of cryptic, systemic Botrytis infections in symptomless hosts. Front. Plant Sci. **7**: 625. doi:10.3389/fpls.2016.00625. PMID: 27242829.

Shoemaker, R.S., and Eriksson, O. 1967. Paraphaeosphaeria michotii. Can. J. Bot. **45**: 1605–1608. doi:10.1139/b67-166.

Tanaka, H., Saikai, T., Sugawara, H., Tsunematsu, K., Takeya, I., Koba, H., and Abe, S. 2001. Three-year follow-up study of allergy in workers in a mushroom factory. Respir. Med. **95**: 943–948. doi:10.1053/rmed.2001.1187.

Tao, C., Li, R., Xiong, W., Shen, Z., Liu, S., Wang, B., and Kowalchuk, G.A. 2020. Bio-organic fertilizers stimulate indigenous soil *Pseudomonas*

populations to enhance disease suppression. Microbiome, **8**: 137. doi:10.1186/s40168-020-00892-z. PMID: 32962766.

Thoms, D., Liang, Y., and Haney, C.H. 2021. Maintaining symbiotic homeostasis: how do plants engage with beneficial microorganisms while at the same time restricting pathogens? Mol. Plant Microbe Interact. **34**(5): 462–469. doi:10.1094/MPMI-11-20-0318-FI.

Van Kan, J.A.L., Shaw, M.W., and Grant-Downton, R.T. 2014. *Botrytis* species: relentless necrotrophic thugs or endophytes gone rogue? Mol. Plant Pathol. **15**(9): 957–961. doi:10.1111/mpp.12148. PMID: 24754470.

Vanam, H.P., Deshpande, R., Nayanagari, K., Sreedhar, V., and Rudramurthy, S.M. 2020. *Nigrospora oryzae* pulmonary infection in a bronchogenic cancer: an opportunistic invader? SN Compr. Clin. Med. **2**: 919–927. doi:10.1007/s42399-020-00340-x.

Vellanki, S., Navarro-Mendoza, M.I., Garcia, A.E., Murcia, L., Perez-Arques, C., Garre, V., and Lee, S.C. 2018. *Mucor circinelloides*: growth, maintenance and genetic manipulation. Curr. Protoc. Microbiol. **49**(1): e53. doi:10.1002/cpmc.53. PMID: 30040216.

Vida, C., de Vicente, A., and Cazorla, F.M. 2019. The role of organic amendments to soil for crop protection: induction of suppression of soilborne pathogens. Ann. Appl. Biol. **176**(1): 1–15. doi:10.1111/aab.12555. PMID: 32139916.

Visioli, G., Lauro, M., Vamerali, T., Dal Cortivo, C., Panozzo, A., Folloni, S., and Ranieri, R.A. 2020. A comparative study of organic and conventional management on the rhizosphere microbiome, growth and grain quality traits of *Tritordeum*. Agronomy, **10**(11): 1717. doi:10.3390/agronomy10111717.

Wang, C.-G., Vlasak, J., and Dai, Y-C. 2021. Phylogeny and diversity of Bjerkandera (*Polyporales*, *Basidiomycota*), including four new species from South America and Asia. MycoKeys, **79**: 149–172. doi:10.3897/mycokeys.79.63908. PMID: 33958953.

Wang, M., Liu, F., Crous, P.W., and Cai, L. 2017. Phylogenetic reassessment of *Nigrospora*: ubiquitous endophytes, plant and human pathogens. Persoonia, **39**: 118–142. doi:10.3767/persoonia.2017.39.06. PMID: 29503473.

Wang, X.W., Yang, F.Y, Meijer, M., Kraak, B., Sun, B.D., Jiang, Y.L., Wu, Y.M., Bai, F.Y., et al. 2019. Redefining *Humicola sensu stricto* and related genera in the *Chaetomiaceae*. Stud. Mycol. **93**: 65–153. doi:10.1016/j.simyco.2018.07.001. PMID: 30210181.

Wery, N. 2014. Bioaerosols from composting facilities – a review. Front. Cell Infect. Microbiol. **4**: 42. doi:10.3389/fcimb.2014.00042. PMID: 24772393.

Xia, Y., DeBolt, S., Dreyer, J., Scott, D., and Williams, M.A. 2015. Characterization of cultural bacterial endophytes and their capacity to promote plant growth from plants grown using organic or conventional practices. Front. Plant Sci. **10**: 490. doi:10.3389/fpls.2015.00490.

Xia, Y., Sahib, M.R., Amna, A., Opiyo, S.O., Zhao, Z., and Gao, Y.G. 2019. Culturable endophytic fungal communities associated with plants in organic and conventional farming systems and their effects on plant growth. Sci. Rep. **9**: 1669. doi:10.1038/s41598-018-38230-x.