

Fungi on Coffee

Subjects: Mycology

Contributor: li LU, Saowaluck Tibpromma, Samantha Karunarathna, Saisamorn Lumyong, Jianchu Xu, Kevin D. Hyde

Coffee is grown in more than 80 countries as a cash crop and consumed worldwide as a beverage and food additive. It is susceptible to fungal infection during growth, processing and storage. Fungal infections, in particular, can seriously affect the quality of coffee and threaten human health.

Keywords: endophytes ; fungal diseases ; fungal toxins ; pathogens ; postharvest diseases

1. Introduction

Coffee has gained in popularity in modern times and is the second-best-selling beverage in the world ^[1]. As an important economic crop, it is central to the livelihoods of millions of people worldwide ^[2]; accordingly, more than 80 countries grow coffee and some countries use coffee as a major cash crop ^[3]. World coffee production for 2020/2021 is forecast to be 5.5 million kg higher than the previous year, reaching a record 176.1 million kg ^[4]. Brazil is the largest exporter of coffee, and its exports account for one-third of the global total ^[5]. The Agricultural Trade Office in Sao Paulo (ATO) forecasts the Brazilian coffee production for 2020/2021 at a record of 67.9 million kg, an increase of 15% over 2019 output. Finland is the largest per capita consumer of coffee, while China consumes the most coffee by volume ^[6]. *Coffea arabica* and *C. canephora* (robusta) are the two most-grown coffee species in the world ^[7], accounting for 60% and 40% of global production, respectively ^[8].

Throughout the tropics, coffee growers face many problems in agricultural production ^[9]. As a climate-sensitive plant, implications of climate change have altered coffee production, from decreasing crop yield and quality to increasing fungal diseases and invasive pests ^[10]. Coffee worldwide suffers from a range of pests and diseases, and fungal infections are also a major problem ^[11]. Coffee roots, stems, leaves and beans are often damaged by pests and pathogens ^[12].

Fungi on coffee occur in different life modes: endophytes, pathogens and saprobes ^{[13][14][15]}. The largest number of fungi have been recorded from *C. arabica* and *C. canephora* (Table S1, supplementary could be found in <https://www.mdpi.com/2076-0817/11/4/411#supplementary>, the following supplementary is same). Endophytes usually live inside the host without causing injury or obvious symptoms, and this association can provide a better living environment for both the host and fungus ^[16]. There are also reports on their ability to aid in the defense of host plants ^{[17][18]}. Huang et al. ^[19] screened potential antagonistic endophytes that prevent and control post-harvest diseases. Coffee easily can be infected by pathogenic fungi when growing, during post-harvest handling and storage, and during processing ^[20]. One of the most virulent diseases is 'coffee rust' caused by *Hemileia vastatrix*, which wiped out coffee 150 years ago and continues to cause problems in coffee plantations worldwide ^{[21][22]}. Fungal diseases in coffee can be divided into two types: diseases in pre-harvest and diseases in post-harvest ^{[23][24]}. Many post-harvest coffee pathogens are infected shortly before harvest, are generally not found at harvest, and feature low activity; moreover, poor storage conditions during post-harvest favour their development ^[23]. Fungal invasions before harvest are mainly induced by the interaction between the plant host and other organisms (such as insects), while fungal infections after harvest are controlled by nutrient availability, temperature, humidity and biological factors (insects) ^[25]. Another pathway is that endophytic fungi in coffee beans change their life modes to saprobic/pathogenic after the beans are harvested, becoming postharvest pathogens ^{[19][26]}. Most postharvest fungi produce toxins as secondary metabolites viz. ochratoxin-A, which is a mycotoxin mainly produced as a result of secondary metabolism of many species of *Aspergillus* and *Penicillium* and is the most common mycotoxin present in agricultural commodities ^[27]. Toxin-producing fungi can be isolated from coffee beans both pre-harvest and post-harvest, while the risk of fungal growth and mycotoxin production after harvest is higher in high temperature areas ^{[28][29]}. These toxins can cause host infections and reduce coffee bean quality ^[30] and can be carcinogenic, hepatotoxic, hematotoxic, nephrotoxic and neurotoxic for humans ^{[31][32]}. Silva et al. ^[33] isolated ochratoxin-A from damaged coffee beans, and ochratoxin-A was shown to cause coffee quality and yield losses. Studies have shown that the main toxigenic fungal genera comprise *Aspergillus*, *Penicillium* and *Fusarium*, which are natural coffee contaminants ^[34], and they can infect hosts in both farms and warehouses ^[35].

2. Records of Coffee Fungi

A total of 966 records of coffee fungi ([Supplementary Table S1](#)) were found in the literatures, belonging to 113 genera of which frequently found families and genera are shown in **Figure 1a** and [Supplementary Table S2](#).

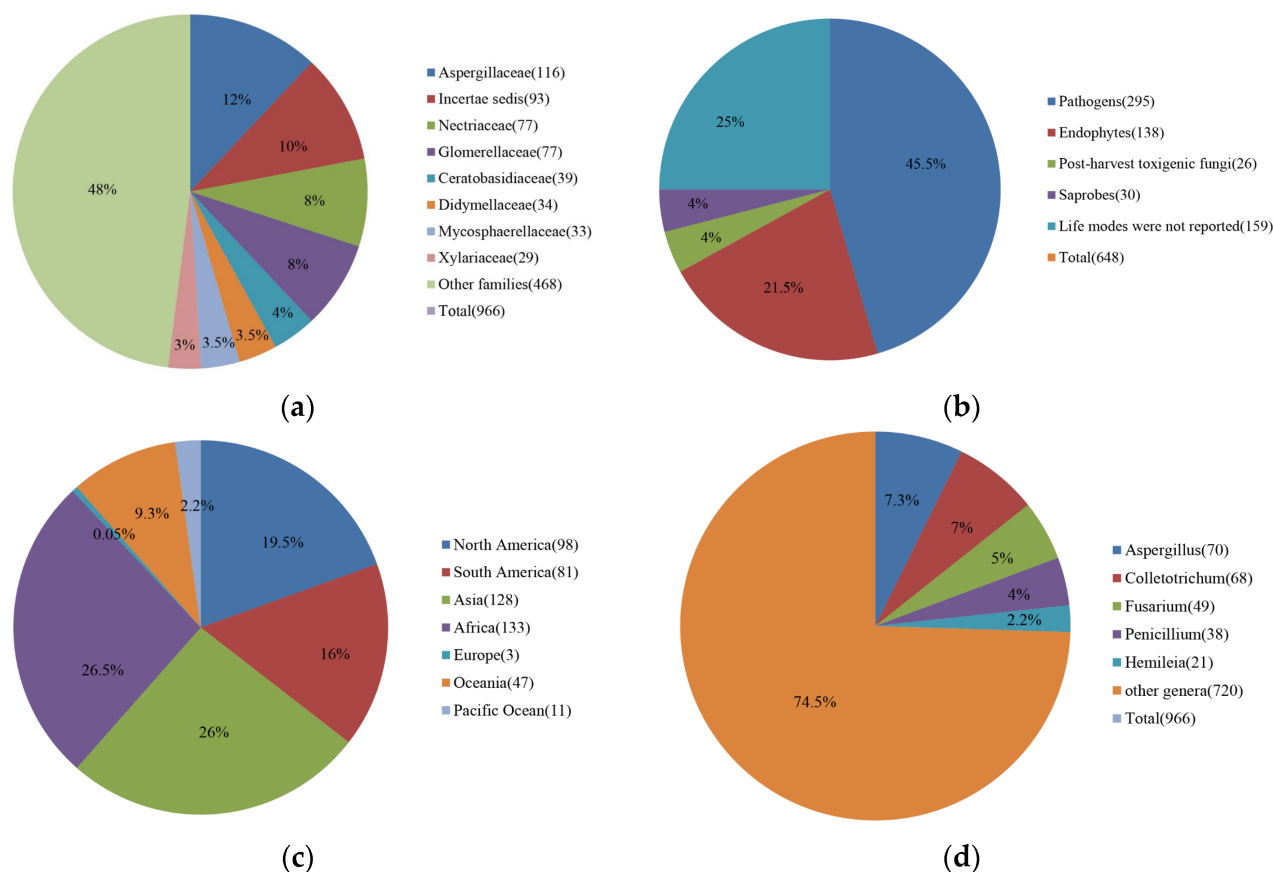


Figure 1. (a) Percentages of different fungal families reported on coffee; (b) Percentages of different life modes of fungi reported on coffee; (c) Percentages of coffee pathogens reported in different countries; (d) Percentages of most frequently reported fungal pathogenic and toxigenic fungal genera on coffee.

Out of 648 fungal species, 295 are pathogenic, 138 are endophytes, and 30 are saprobies, while the life modes of 159 species have not been confirmed and another 26 species are post-harvest disease causing agents that can produce mycotoxins in dried and green coffee beans (**Figure 1a,b**). It was not confirmed in reported publications whether most reported pathogens caused pre-harvest or post-harvest infections. Of the 295 species of pathogens, 212 species are true pathogens (TP), three species are post-harvest pathogens (PP) and four species are both true pathogens (TP) and post-harvest pathogens (PP), while the other 76 species are listed as unknown (UNK) as their disease symptoms have not been confirmed on coffee ([Supplementary Table S4](#)).

3. Endophyte Role in Coffee Plants

Endophytic fungi that can inhibit nematodes, coffee berry borers and pathogenic fungi have the potential to be used as biocontrol agents to control pest and pathogen infections of coffee [36][37][38]. Goates et al. [39] showed that some endophytic fungi reduce fungal diseases by producing volatile organic compounds that can kill or inhibit phytopathogens. Monteiro et al. [40] demonstrated that volatile organic compounds produced by the endophyte *Muscador coffeanum* isolated from *C. arabica* produce fungicidal activity against *Aspergillus ochraceus*. Three records of *Muscador* species belonging to induratiaceae [41] were found in coffee according to researchers' data. De Almeida et al. [42] isolated *Aspergillus* sp., *A. westerdijkiae*, *A. niger*, *A. tamari*, and *A. fumigatus*, *Lichtheimia ramosa* and *Rhizopus oryzae* from coffee beans to test their abilities to inhibit the growth of *Aspergillus* species and ochratoxin-A production, and *A. niger* showed the best inhibitory ability of both growth and ochratoxin-A production. According to researchers' results, Aspergillaceae is the most frequently found family in coffee. Furthermore, Eida et al. [43] isolated *Penicillium crustosum*, *Penicillium verruculosum*, *Trichoderma harzianum*, and *Hypocrea lixii* from coffee residue compost that can aid in the degradation of lignocellulose waste.

4. Pathogen Effect on Coffee and Coffee Disease

Among 648 species, fungal pathogens are the most common (295 species) as shown in **Figure 1b**. According to Supplementary Tables S1 and S3: Sixty-eight of *Colletotrichum* belonging to 35 species have been reported. Coffee production is often affected by Coffee Berry Disease, which is the main factor limiting the production of *C. arabica* in Kenya and other countries in East Africa, especially in high-altitude areas. It is potentially responsible for 50 to 80% of total crop losses ^[44]. Forty-nine *Fusarium* belonging to 23 species have been reported (Supplementary Tables S1 and S3). *Fusarium* species are one of the most important phytopathogenic and toxin-producing fungi ^[45]. Coffee Wilt Disease is a devastating disease in East and Central Africa ^[46]. Coffee Wilt Disease is a vascular disease, and due to its high transmission potential, Coffee Wilt Disease poses a threat to all coffee-producing regions. This disease can kill its host at all ages in a short time ^[47]. Moreover, after the infected trees and their roots are removed, the infested soil may remain infectious for several years ^[48]. Twenty-one *Hemileia* have been reported belonging to two species, and *Hemileia vastatrix* is an important phytopathogenic fungus that causes Coffee Leaf Rust. Coffee Leaf Rust, one of the major diseases of Arabica coffee is a major threat to coffee production worldwide and it has been reported to cause serious economic losses in more than 50 coffee-growing countries ^[49]. Brown Eye Spot or *Cercospora* Blotch caused by *Cercospora coffeicola*, has been reported on coffee. Besides, Andrade et al. ^[50] showed that isolates producing brown eye spot and black spot can also cause prompt alterations in the antioxidant metabolism of coffee leaves. *Armillaria* root rot caused by *Armillaria* sp., has been reported on coffee, and this disease leads to coffee plant rot and the eventual death of the plant. Since this fungus invades deeper into roots, symptoms are difficult to detect, thus it can last for several years before symptoms appear on the surface. This disease spreads to other plants with the transfer of soil ^[51]. Major coffee diseases that have a huge impact on coffee plantations worldwide are discussed in detail below ^[14].

4.1. Detection and Identification of Diseases

Detection and identification of fungal diseases in crops can be done through direct and indirect methods. Direct detection of fungal diseases includes molecular and serological methods, while indirect methods identify the plant diseases through various parameters such as morphological change, temperature change, transpiration rate change, and volatile organic compounds released by infected plants ^[52]. Among different methods, fungal morphology is a commonly used method to identify coffee pathogens ^[11], while pathogenicity tests ^[53] and Polymerase Chain Reaction (PCR) ^[54] are also used. Generally, three main methods are used for the identification of coffee fungal pathogens.

Fungal morphological characteristics—Different fungal pathogens cause different symptoms on the host surface ^[14]. Firstly, the disease symptoms on the host are observed and recorded, and then the pure cultures grown on potato dextrose agar (PDA) are obtained according to the isolation method of Senanayake et al. ^[55]. Finally, colony size, colour of the conidial masses and zonation, size, and shape of conidia harvested from the cultures are recorded under the microscope ^[44].

Pathogenicity test (Koch's postulates)—Spore suspensions of pathogenic fungi are obtained by pure cultures grown on PDA for 7 to 10 days, prepare healthy/disease-free hosts, then carry out the pathogenicity test ^[44]. After inoculation, the changes are recorded from 1 to 15 days of growth, compare the morphological characteristics of the lesions in the host with original disease lesions.

Polymerase Chain Reaction—Pure cultures of pathogenic fungi grown on PDA for 7 to 10 days are used to scrape mycelium from the culture surface. Then, the genomic DNA is extracted using a Genomic DNA Extraction Kit or CTAB. Finally, PCR amplification is done for the specific genes of interest ^[44].

4.2. Coffee Leaf Rust

- Pathogen: *Hemileia vastatrix* has spread to all coffee cultivation areas worldwide. *Hemileia coffeicola* is restricted to central and western Africa, especially in higher and cooler regions ^{[22][56]}.
- Hosts: *C. arabica* (arabica coffee) and *C. canephora* (robusta coffee), the two most important commercial coffee species ^[56].
- Symptoms and signs: Infection occurs on the leaves of coffee. The first observable symptoms are small, and light-yellow spots on the upper surface of leaves. As the diameter of these points gradually increases, a large number of orange urediniospores (=uredospores) appear under the leaf surface. The fungus forms spores through stomata instead of penetrating the epidermis like most rust-causing species, so it does not form many typical rust pustules. Powdery lesions under leaves appear orange-yellow to red-orange with a high degree of variance. Although disease

spots can develop anywhere on the leaf, they tend to concentrate around the edges, where dew and raindrops gather. The center of the spot eventually dries out and turns brown, while edges of the lesions continue to expand and produce new spores. At the beginning of the season, the disease usually first appears on the lower branches, and infection progresses slowly up the tree. Infected leaves fall prematurely, leaving long branches without leaves [14][56][57][58][59].

- Pathogen biology: *Hemileia vastatrix* mainly exists in the form of dikaryotic, and nutrient-absorbing mycelium between cells in leaves of its coffee host. Short pedicels are clustered throughout stomata and below leaves, with dual-nucleated spores. Towards the end of the season, sometimes under cool, dry conditions, spores are produced from polyspores on older attached leaves. After nuclear division and meiosis, these sporozoites germinate to produce basidia, each of which forms four haploid sporozoites [14][56][57][58][59].
- Disease cycle: Urediniosporic life cycle as its most important source of inoculum, can cause infection and develop into lesions, producing more urediniospores. Spore adhesion to the host surface, germination of urediniospores, formation of an adhesion layer on stomata, penetration, and intercellular and intracellular colonization are various steps of the disease cycle. The disease cycle of Coffee Leaf Rust is discussed in Talhinhos et al. [22].
- Disease management:
- Chemical control: Fungicide sprays (Epoconazole, Pyraclostrobin, Cyproconazole, Hexaconazole or Cupric Fungicides) [14][22].
- Cultural practices: Agroforestry practices of tree-crop mixing, timely pruning, handling and de-suckering, regular change of crop cycle [49].
- Biological practices: *Pichia membranifaciens* is a yeast strain isolated from soil that can reduce the *Hemileia vastatrix* spore viability [59].
- Resistant varieties: Such as HDT (Híbrido de Timor), Catimor and Sarchimor populations [22].

4.3. Coffee Berry Disease

- Pathogen: *Colletotrichum kahawae* is a particularly devastating pathogen that affects developing berries, leading to berry rot and shedding before bean formation. *Colletotrichum kahawae* has not been reported outside Africa or in low altitudes. Coffee Berry Disease was first detected and identified by McDonald in Kenya in 1922 [14].
- Hosts: Mainly *C. arabica* (arabica coffee) [14].
- Symptoms and signs: Characteristic symptoms are progressive anthracnose of young and expanding coffee berries. Symptoms present as small water-soaked lesions that rapidly become dark and sunken. These lesions expand, causing rot of the entire berry under humid conditions, and pink spore masses become visible on the lesion surface. Berries are often shed from branches at an early stage of the disease. Lesions may also occur on young berry stalks, causing them to shed before lesions are evident on berries. Pale, corky lesions (scab lesions) also appear on young and mature berries that are resistant to infection. They may completely heal or remain dormant until berries ripen. This disease also affects ripening berries, causing a 'brown blight' phase as typical dark, sunken anthracnose lesions that envelop the red berries. *Colletotrichum kahawae* may also infect flowers under wet conditions, causing brown lesions on petals [14][58][60].
- Pathogen biology: The fungus settles in the mature bark of coffee buds and infects flowers, mature fruits, and leaves. Under high humidity and high temperature, conidia germinate and form germ tubes and appendages when contacted with susceptible tissues [61].
- Disease cycle: The Coffee Berry Disease cycle begins each year at the first rain event and is subsequently maintained by rain-splash dispersal and secondary inoculation of conidia onto healthy berries in the rainy season. The disease cycle of Coffee Berry Disease is discussed in De Silva et al. [62].
- Disease management:
- Cultural practices: Shading with fruit trees and irrigation to induce early flowering to decrease the severity and all berries should be removed at the end of the planting season to prevent them from becoming a source of inoculation for new crops [60].

- Biological control: Many components in the microbiota (fungi and bacteria) on coffee trees show very high antagonistic levels and have a strong antagonistic effect on *Colletotrichum kahawae*. However, these agents have not been developed into commercial biocontrol agents [60].
- Chemical control: The most economical method is to use a mixture of copper fungicide (50% wettable copper chloride wettable powder 5 kg) and organic fungicide (75% chlorothalonil wettable powder 2 kg) [14][60].
- Resistant varieties: Ruiru 11, Hibrido de Timor, Rume Sudan, K7, and several Catimors. In Ethiopia, 37 Coffee Berry Disease resistant coffee cultivars are used [14][60].

4.4. Coffee Wilt Disease

- Pathogen: *Fusarium xylarioides* causes wilt in *Coffea excelsa* (*C. liberica*) (Steyaert 1948). This disease was first detected in 1927 in Oubangui-Chari (now the Central African Republic) and was initially thought to be caused by a root rot [14][47].
- Hosts: *C. arabica* (arabica coffee), *C. canephora* (robusta coffee), and *C. excelsa* [47].
- Symptoms and signs: First, leaves turn yellow before withering and developing brown necrotic lesions. Finally, leaves curl, dry, and fall. This process can start from any part of the plant, but eventually, symptoms spread to the rest of the plant. Symptoms first present on the coffee stem, where fungi colonize, and the host response blocks vascular bundles, resulting in blue-black stains [14][47][58][63].
- Pathogen biology: Conidia and ascospores are spread by wind, rain and through human activities (harvesting, pruning). Pathogens penetrate wounds, so any agent causing wounds aids the spread of the fungus. Krantz and Mogk in 1973 noted that most dying and dead trees had been wounded during weeding. Insects may also spread the disease from one tree to another tree [64].
- Disease cycle: Incubation period from first symptoms to death of tree varies, although most affected trees die 2–3 months after initial symptoms were observed. It usually quickly kills infected mature trees within just 6 months after the first external symptoms appear, resulting in a decline of total yield. Coffee quality may also be affected by premature berry ripening. The disease cycle of Coffee Wilt Disease is discussed in Alemu et al. [65].
- Disease management:
- Cultural practices: Frequent inspection, along with burning infected material and spraying soil surfaces with 2.5% copper (II) sulphate. Replanting should not be done until 6 months after uprooting infected trees to allow the viability of soil inoculum to decline. It is recommended to grow cover crops such as *Desmodium* sp. and haricot bean, which are very efficient in suppressing weeds (so reducing the need for slashing) and as legumes, promote the growth of coffee trees [63].
- Chemical control: Ridomil Gold (metalxyl 8% + Mancozeb 64%) 68% Wp 2.5 kg/ha, when disease on set, used at 7, 14, 21, 28 days. Pencase 80% WP (Mancozeb) at the rate of 2.5 kg/ha, when disease on set, used at 7, 14, 21 days [63].
- Biological control: The strain of *Bacillus subtilis* (AUBB20) is the most antagonistic to this disease. *Trichoderma viride* and *Trichoderma harzianum* have shown good potential in inhibiting the mycelial growth of *Fusarium xylarioides*, but no effective methods of biological control are currently available [63].

4.5. Brown Eye Spot or *Cercospora* Blotch

- Pathogen: *Cercospora coffeicola* is distributed throughout the tropics and subtropics and is prone to appear on coffee plants in areas with higher moisture and rainfall as well as on plants that are stressed [14][66].
- Hosts: *C. arabica* (arabica coffee), *C. canephora* (robusta coffee) [66].
- Symptoms and signs: on the leaves, small, round to irregular spots, and brown to light brown lesions first appear. The number and size of lesions then increase before eventually the entire leaf is affected. The edge of the lesion may appear dark purple or black, and it may be encircled by a yellow halo. Severely infected leaves turn yellow and fall off; lesions on green berries are initially brown, sunken, longitudinal, irregular or oval with a gray center. Infection can occur at any stage of berry growth; on the red cherries, first, large, sunken, and blackened areas cover with silvery fungal

- spores. Penetration into the seeds may cause the pulp to stick to parchment paper during processing, and damage the product. *Cercospora coffeicola* reduces productivity and lowers the beverage quality of coffee [14][57][67].
- Pathogen biology: Wind, splashing water and human activities cause spores (conidia) to be deposited on leaves and petioles, beginning the disease cycle. Conidia germinate at moderate to warm (20–28 °C) temperatures [68].
 - Disease cycle: In warm and humid periods, new infections and sporulation occur every 7 to 10 days. Pathogen easily spreads in fields via wind, rain, and irrigation water. It survives as a pathogen in weeds and infested crop fragments, where it is capable of re-infecting grown plants. The disease cycle of *Cercospora* Blotch is discussed in Souza et al. [69].
 - Disease management:
 - Biological control: No biological control measures have been developed [68].
 - Cultural practices: Elimination of crop debris, weed hosts and provide 35–65% shade. In order to maintain adequate plant nutrition, nitrogen fertilizers are used. Plant only high-quality seeds, and destroy infected crops in time after the final harvest and before replanting. Select a reasonable planting density (10 ft × 10 ft for robusta while 8 ft × 8 ft for arabica). Avoid planting coffee transplants too deep in soils [68].
 - Chemical control: Fungicide sprays are necessary for disease control in wet conditions, but proper fungicides, rates, and fungicide rotations such as Chlorothalonil and Chlorothalonil Mixtures, Strobilurins and Strobilurin Mixtures should be followed [68].

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