



Isolation and Identification of Mycotoxin-Producing Fungi in Raw Coffee

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I. Abstract

This study aimed to isolate and identify mycotoxin-producing fungi in raw coffee beans and to evaluate the chemical properties of the coffee. The results indicated significant differences ($p < 0.05$) in the studied characteristics, including the chemical composition of the coffee bean samples. Moisture content ranged from a maximum value of 12.53% in treatment T6 to a minimum value of 9.78% in T5 at a significance level of $p \leq 0.05$, with T3 and T6 recording 11.48% and 12.53%, respectively. Regarding protein content, T4 exhibited a significant superiority ($p \leq 0.05$), recording 13.59%. For lipid content, T4 surpassed the other treatments, registering the highest value. As for ash content, T4 recorded 5.11% at a significance level of $p \leq 0.05$, while the lowest ash content was observed in T3 at 4.33%.

Furthermore, various fungal species isolated from the coffee beans were identified. *Aspergillus flavus* was the most prevalent with an isolation frequency of 83.33%, followed by *Aspergillus ochraceus*, *Aspergillus carbonarius*, and *Aspergillus westerdijkiae* at a frequency of 66.67%. The isolation frequency for *Penicillium verrucosum*, *Aspergillus parasiticus*, *Aspergillus steynii*, and *Aspergillus fumigatus* reached 33.33%, whereas the frequency for *Aspergillus melleus*, *Aspergillus tubingensis*, *Mucor* spp., and *Rhizopus* spp. was 16.67%.

Keywords : Coffee, Fungi, Mycotoxins



II. Introduction

Coffee is a fundamental element of the global economy and holds a prominent position among the most consumed beverages worldwide. It ranks as the second most traded commodity after oil, providing substantial foreign exchange revenues for many developing nations. Over 125 million people worldwide are employed in the cultivation, processing, and marketing of coffee. Its economic impact is particularly pronounced in developing countries, where coffee exports account for more than 50% of foreign exchange earnings (Jiménez & Carril, 2014).

Coffee is primarily derived from two species: Arabica coffee (*Coffea arabica*) and Robusta coffee (*Coffea canephora*). Arabica coffee represents approximately 75% of the global market, while Robusta coffee accounts for the remaining 25% (Liu, 2022). Fungal contamination in coffee beans is significantly influenced by a variety of environmental and technical factors, which collectively create favorable conditions for fungal growth and mycotoxin production. Climatic conditions during the growth and harvest stages, such as temperature and humidity, are critically important as they directly affect the proliferation of fungi like *Aspergillus*, *Penicillium*, and *Fusarium*, which are common contaminants in coffee beans (Lu et al., 2022).

Various strategies have been explored to mitigate these contaminants, including roasting, microwave treatment, ultraviolet irradiation, and the application of nanoparticles. Studies have demonstrated that conventional coffee roasting reduces mycotoxin levels, with research indicating a reduction of up to 64.70% for Ochratoxin A and 62.38% for Aflatoxin B1, depending on the roasting method and degree (Al Attiya et al., 2021). However, roasting alone may not be sufficient, as some degradation products of Ochratoxin A remain nephrotoxic (Cruz & Casal, 2019). The efficacy of microwave and ultraviolet irradiation treatments in mycotoxin decontamination has also been investigated.

Based on the aforementioned, this study aimed to isolate contaminating fungi from raw coffee beans obtained from various sources; identify the isolated fungi relying on phenotypic and microscopic characteristics; conduct genetic characterization of mycotoxin-producing fungi using molecular techniques; and estimate the levels of aflatoxins and Ochratoxin A in the raw coffee samples.

III. Materials and Methods

Culture Media: Culture media were prepared, and all equipment used was sterilized to ensure the absolute absence of microbial contamination, strictly following the manufacturers' instructions.

Sample Collection: This study was conducted on six types of coffee beans sourced from various origins, representing the most commonly consumed varieties in the Iraqi market. The samples were purchased as raw beans, preserved in clean, tightly sealed nylon bags, and subsequently stored in a laboratory refrigerator until the required experiments were conducted. Each bag was labeled with an identification tag detailing the product name and country of origin prior to undergoing the necessary analyses, as outlined in Table (1-1).

Table (1-1): Coffee samples under study

Sample Code	Origin (Coffee Type)	Species (Variety)
T1	Brazilian coffee	Arabica
T2	Indian coffee	Arabica
T3	Colombian coffee	Arabica
T4	Indonesian coffee	Arabica
T5	Vietnamese coffee	Arabica
T6	Ethiopian coffee	Arabica

Fungal Cultivation The direct plating method is one of the most common and practical techniques for isolating and identifying fungi that contaminate grains and their products. In this study, a chlorine solution of 0.2% sodium hypochlorite was used to wash and surface-sterilize the coffee bean samples to eliminate external contamination. Subsequently, the samples were washed with sterile distilled water with vigorous shaking to ensure the complete removal of chlorine solution residues. They were then left to dry under aseptic conditions for 15 minutes. Five beans from each sample were transferred and inoculated onto the prepared, solidified culture media in Petri dishes.

The plates were incubated at 25°C for five days to monitor fungal growth and facilitate subsequent identification.

Isolation and Identification of Fungi The growing fungi were subcultured onto Potato Dextrose Agar (PDA). Upon the completion of the incubation period, the isolated fungi were identified morphologically and examined microscopically according to the standard methods outlined by AOAC (2005).

Chemical Evaluations of Coffee

- **Determination of Fat Percentage** Fat content was determined using a Soxhlet extraction apparatus

, according to the method described by A.O.A.C (2005). The fat percentage in the sample was calculated using the following equation:

Determination of Protein The protein percentage in the samples was determined using the standard Kjeldahl method

, based on the methodology described by A.O.A.C (2005).





Statistical Analysis Data were statistically analyzed using the experimental system within the SAS (2001) statistical software package. A Completely Randomized Design (CRD) was employed, and the means were compared using Duncan's multiple range test (Duncan, 1955).

IV. Results and Discussion

Chemical Composition of Coffee

The results regarding the chemical composition of the coffee bean samples, presented in Table (1-2), indicated significant differences among the studied characteristics. Moisture content ranged from a maximum value of 12.53% in T6 to a minimum value of 9.78% in treatment T5 at a significance level of $p \leq 0.05$, while T3 and T6 recorded 11.48% and 12.53%, respectively. It is noteworthy that these levels fall within the internationally established moisture limits for green coffee beans. Regarding protein content, T4 exhibited a significant superiority ($p \leq 0.05$), recording 13.59%, whereas T5 registered the lowest value at 10.79%. For lipid content, T4 surpassed the other varieties in this study, recording the highest value of 12.15%, while the lowest value was observed in T1 at 10.10%. As for ash content, T4 recorded 5.11% at a significance level of $p \leq 0.05$, while the lowest ash percentage was found in T3 at 4.33%.

The critical moisture content in coffee, which typically ranges between 10% and 12%, is paramount for maintaining its quality. Exceeding this limit can lead to increased water activity, a crucial factor in the metabolic activity of microorganisms and fungi. Therefore, maintaining moisture content within this optimal range minimizes the risk of fungal contamination and preserves coffee quality during storage. Studies have demonstrated that coffee beans with moisture levels within the recommended range exhibit lower water activity values, rendering them less susceptible to spoilage (Dos Santos et al., 2020). The results for treatments T1 through T5 were consistent with or below the ideal range (10% - 12%), as specified by Osorio et al. (2024). In contrast, sample T exceeded this limit significantly, reinforcing the hypothesis of elevated water activity in this sample, which aligns with the findings of Santos et al. (2020). Generally, maintaining moisture content within the optimal range is essential to ensure the safety and quality of coffee, prevent fungal growth, and preserve its sensory properties during storage and processing (Saath et al., 2012).

Regarding the determination of protein percentage in the studied coffee samples, the recorded values indicated a strong agreement with previous studies. Rawel and Sagu et al. (2023) reported that the protein content in green coffee beans typically ranges between 11% and 13% on a dry weight basis. The stability of the results for treatments (T1, T2, T3, T4, T6) within this range reflects the high quality of the beans used. The presence of sample T4 at the upper limit (13.59%) indicates a high structural quality of the beans, making them an ideal reservoir for essential amino acids. Conversely, the slight decrease in sample T5 is attributed to environmental factors or effects related to storage and processing conditions, a finding consistent with Samsudin and Syafaruddin (2022).

The lipid content in the coffee samples at these proportions in the "raw state" reflects the integrity of the beans and the preservation of intact lipid bodies within the endosperm tissue. This stability proves that the beans have not undergone deterioration or rancidity, providing a solid baseline for comparison. These results are in agreement with the findings



of Speer and Kölling (2006). Furthermore, lipid composition can be influenced by factors such as the geographical origin of the coffee, processing methods, and storage conditions, which may ultimately affect the quality of the coffee beverage (Echeverri-Giraldo et al., 2020).

Ash represents the inorganic mineral components (such as potassium and magnesium) absorbed by the tree from its environment. The mineral content of raw coffee beans, including ash, constitutes approximately 4% of their dry weight, a percentage influenced by factors including processing methods and geographical origin (Cruz et al., 2015). Elemental composition, including ash content, serves as a useful indicator for determining the authenticity and origin of coffee, as it reflects the unique elemental patterns associated with the soil and environmental conditions where the coffee is cultivated (Pohl et al., 2013). Given that the ash content in green coffee beans typically ranges between 3% and 5%, this study agrees with the findings of Morgano et al. (2002), who analyzed 45 samples of Brazilian coffee. Since the analytical percentages in our study fell within these limits, they are consistent with the previous studies cited.

Table (1-2): Chemical composition of coffee beans (Mean \pm Standard Error)

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
T1	10.14 \pm 0.08 d	11.24 \pm 0.70 c	10.10 \pm 0.20 d	4.29 \pm 0.11 d
T2	11.11 \pm 0.07 b	11.23 \pm 0.28 c	11.22 \pm 0.04 b	4.70 \pm 0.09 bc
T3	11.48 \pm 0.12 a	12.17 \pm 0.10 b	10.43 \pm 0.06 c	4.33 \pm 0.08 d
T4	10.38 \pm 0.08 c	13.59 \pm 0.90 a	12.15 \pm 0.50 a	5.11 \pm 0.60 a
T5	9.78 \pm 0.07 e	10.79 \pm 0.10 d	10.34 \pm 0.40 cd	4.54 \pm 0.50 cd
T6	12.35 \pm 0.16 a	13.35 \pm 0.10 a	10.22 \pm 0.06 cd	4.89 \pm 0.07 ab

Isolation and Identification

The results presented in Table (1-3) revealed various fungal species isolated from the coffee beans. *Aspergillus flavus* predominated with an isolation frequency of 83.33%, followed by *Aspergillus ochraceus*, *Aspergillus carbonarius*, and *Aspergillus westerdijkiae* at a frequency of 66.67%. Meanwhile, the isolation frequency of *Penicillium verrucosum*, *Aspergillus parasiticus*, *Aspergillus steynii*, and *Aspergillus fumigatus* reached 33.33%, whereas the frequency of *Aspergillus melleus*, *Aspergillus tubingensis*, *Mucor* spp., and *Rhizopus* spp. was 16.67%.

The diversity of fungi within the coffee samples serves as clear evidence that the coffee was exposed to unfavorable environmental conditions during the production, harvesting, and storage stages, which subsequently facilitated the growth of these fungal species. Such conditions include elevated moisture levels and temperatures optimal for their proliferation, in addition to prolonged storage periods.

These findings are in agreement with Nogaim (2013), who found that *Aspergillus flavus* recorded the highest frequency of 83.33% in coffee samples. This aligns with numerous studies indicating that this species is among the most widespread fungi in stored agricultural products, primarily due to its high capacity to adapt to environmental stress conditions and low water activity. Furthermore, this fungal species is a known producer of aflatoxins, which significantly heightens the health risks associated with coffee contamination. The results also concurred with Aasa

(2021), who reported that the isolation frequency of *Aspergillus ochraceus*, *Aspergillus carbonarius*, and *Aspergillus westerdijkiae* reached 66.67%. These specific fungi are well-recognized for their high capacity to produce Ochratoxin A , a hazardous mycotoxin with pronounced toxic and potential carcinogenic effects.

Table (1-3): Fungi grown on coffee bean samples

Fungi	T1	T2	T3	T4	T5	T6	Number of Positive Samples	Isolation Frequency (%)
<i>Aspergillus flavus</i>	+	+	+	+	+	-	5	83.33
<i>Aspergillus ochraceus</i>	+	+	+	-	-	+	5	66.67
<i>Aspergillus carbonarius</i>	+	+	-	+	-	+	4	66.67
<i>Aspergillus westerdijkiae</i>	+	+	-	+	-	+	4	66.67
<i>Aspergillus niger</i>	-	-	+	-	+	-	2	33.33
<i>Penicillium verrucosum</i>	-	-	-	+	-	+	2	33.33
<i>Aspergillus parasiticus</i>	-	+	-	-	-	+	2	33.33
<i>Aspergillus steynii</i>	-	+	-	-	-	+	3	50.00
<i>Aspergillus fumigatus</i>	-	-	-	+	-	+	2	33.33
<i>Aspergillus melleus</i>	-	-	-	-	-	+	1	16.67
<i>Aspergillus tubingensis</i>	-	-	-	-	-	+	1	16.67
<i>Mucor spp.</i>	-	-	-	-	+	-	1	16.67
<i>Rhizopus spp.</i>	-	-	-	-	+	-	1	16.67

Morphological and Microscopic Identification

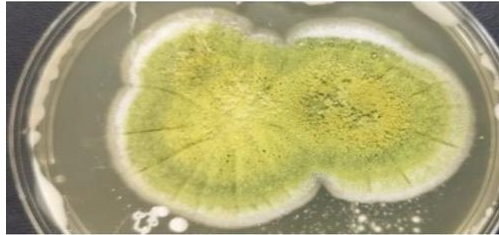


Figure (1) illustrates an image of the fungus *Aspergillus flavus*

The morphological identification of the fungi contaminating the coffee beans revealed the presence of *Aspergillus flavus*, which was characterized by the appearance of green to yellowish-green colonies surrounded by a white halo. The spore heads were compact and spherical with a granular appearance, a pale yellow basal color, and a velvety to cottony texture exhibiting a powdery appearance due to the production of conidiospores. The results also demonstrated rapid growth on Potato Dextrose Agar (PDA) medium at a temperature of $(25 \pm 2)^\circ\text{C}$ over a period of 3-5 days. Morphological examination of this fungus revealed that it possesses conidiophores that are swollen at the apex, bearing long, dry chains, and the conidia are spherical with a diameter ranging between 300-400 micrometers. These results are in agreement with the findings of Seerat et al. (2022), who reported the growth of several fungi on coffee beans, including *Aspergillus flavus*.

Furthermore, the results of the morphological identification revealed the growth of *Aspergillus carbonarius* on the studied coffee beans cultured on PDA medium. The colonies were characterized by a dark color tending towards black, in addition to a high density of spore heads. The colonies exhibited a circular shape with distinct, smooth edges and a dense, velvety texture, while the basal color was relatively lighter. The results also indicated that the colony texture was velvety due to the dense conidiospores. These findings aligned with those of Ismail (2017), who isolated this fungus from various types of coffee beans.



Figure (2): *Penicillium verrucosum* fungus



The colonies growing on the coffee bean samples also indicated the growth of *Penicillium verrucosum*. The fungal colonies appeared green to olive-green, featuring narrow white margins and a velvety texture, with the reverse side of the colonies turning yellow. Microscopically, it possessed rough-walled conidiophores terminating in smooth, spherical conidia arranged in long chains borne on brush-like structures. These results correspond with the findings of Couto et al. (2003) regarding the ability of this fungus to grow on coffee beans.

Based on the growth of the isolates on culture media, colonies of *Aspergillus ochraceus* were observed. They exhibited a yellow to orange color, with white margins during the initial stages of growth. The texture was dense cottony to granular, and the reverse side of the plates was yellowish-brown, approximating a golden color (Pitt & Hocking, 2009). Microscopic identification revealed that the fungus possesses long conidiophores with smooth to rough walls, and contains spherical spore heads bearing conidia (Klich, 2002).

Identification of the fungi grown on the coffee bean samples also showed the growth of *Aspergillus westerdijkiae*. It presented a pale yellow to golden color with regular white edges and a granular texture ranging from light gray to gray, whereas the reverse side of the colony appeared yellowish-brown. This is consistent with the findings of Frisvad et al. (2013). Microscopically, long conidiophores with smooth to rough walls were observed, terminating in large spherical vesicles. Additionally, small, smooth-walled, spherical to sub-spherical conidia were seen. These observations align with those reported by Pivka et al. (2025).

The results of the morphological identification also revealed the growth of *Aspergillus niger* on the studied coffee beans cultured on PDA. Its colonies were initially yellowish-white and subsequently turned black. They were characterized by rapid growth and a granular texture, with a pale yellow reverse side. The colonies possessed long conidiophores with smooth walls ending in massive spherical vesicles, and contained biseriate spore heads. These results are in agreement with Jaikel-Viquez et al. (2025), who isolated this fungus from coffee beans.

Furthermore, colonies of *Aspergillus parasiticus* appeared yellowish-green to dark green with a granular texture. The margins were distinguished by a white color surrounding the colony, and the reverse side was white to pale cream. These results concurred with the findings of Sukmawati et al. (2018). The microscopic description also revealed that they contain short, rough-walled conidiophores terminating in spherical vesicles, and short spore heads covering most of the vesicle's surface. These heads produce distinctive, rough-walled, spherical conidia. These results are consistent with the findings of Diba et al. (2007).





V. References

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