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Postharvest treatment to control mycotoxin in coffee bean using radio frequency

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Abstract Analytical results indicated the presence of ochratoxin A (OTA), aflatoxin B1, and total aflatoxins in all coffee bean samples. The concentration levels ranged from non-detectable to 46.40 µg/kg for OTA, 18.73–50.44 µg/kg for aflatoxin B1, and 10.18–31.75 µg/kg for total aflatoxins. All coffee bean samples were contaminated with both total aflatoxins and aflatoxin B1. Radio frequency (RF) treatments evaluated for the reduction of mycotoxins in coffee beans showed that applying RF at 55.00 °C for 180 seconds did not reduce the percentage of inoculated coffee beans to the same level as the non-RF-treated samples. However, high moisture content was associated with increased levels of ochratoxin A, aflatoxin B1, and total aflatoxins.

Keywords: Coffee, Mycotoxin, *Aspergillus* sp., Ochratoxin, Aflatoxin, Radio frequency

Introduction

Coffee belongs to Rubiaceae, and is one of the most important agricultural products of world trade (United States Department of Agriculture, 2020). Coffee bean quality is critical as a result of strict food-safety and the increase in demand. At present many countries have established regulatory controls over the levels of each mycotoxin measured in various agricultural commodities.

Some mycotoxins have harmful effects on animal and human health. Regulatory control over the level of ochratoxin A in Thailand has set the maximum at 10.0 µg/kg in coffee bean (Agricultural Commodity and Food Standards, 2018a,b). The European Union (EU) has established maximum allowable limits for ochratoxin A in imported coffee products: 5.0 µg/kg for roasted coffee and 10.0 µg/kg for instant coffee. While there is currently no EU-wide limit for green coffee beans, some member states such as Italy have

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introduced their own national regulations to control ochratoxin A levels in green coffee. On the other hand, the United States Food and Drug Administration has not limited the level for ochratoxins in any commodity (Taniwaki *et al*., 2019; Bayman and Baker, 2006).

Similar to other agricultural crops, coffee cherries, parchment coffee, and green coffee beans are susceptible to microbial contamination. The primary mycotoxin-producing genera associated with coffee include *Aspergillus*, *Penicillium*, and *Fusarium* (Ismaiel and Papenbrock, 2015). Mycotoxin contamination can occur at various stages of production, including cultivation, harvesting, post-harvest processing, and storage. The presence and severity of contamination are largely influenced by environmental and processing conditions (Djadjiti *et al*., 2020; Sirinunta and Akarapisan, 2015). Among the different mycotoxins identified in coffee, ochratoxin A (OTA) is the most frequently studied and commonly detected (Al-Abdalall and Al-Talib, 2014; Silvaa *et al*., 2020). The fungi responsible for OTA production and their associated toxins pose a significant risk to coffee producers, consumers, and regulatory authorities.

Currently, chemical fumigation remains the primary quarantine treatment used to control insect pests and microorganisms in stored coffee, both for domestic consumption and international trade. In addition to fumigation, several alternative methods have been explored, including temperature and humidity control, modified atmosphere treatments, irradiation, and plasma treatment (Liu *et al*., 2020). However, these methods often present limitations, particularly in terms of cost and practicality (Zhou *et al*., 2015). As a result, alternative, non-chemical approaches are being investigated to replace traditional fumigation.

Radio frequency (RF) heating, which utilizes electromagnetic waves in the range of 3 kHz to 300 MHz, is one such promising method. In contrast, microwaves occupy the frequency range from 300 MHz to 300 GHz. Specific RF frequencies, such as 13.56, 27.12, and 40.68 MHz, have been designated for industrial, medical, and scientific applications (Komarov *et al*., 2005). Despite its potential, RF heating has been underexplored in the context of coffee bean processing.

The objectives were to evaluate the presence of ochratoxin A, total aflatoxins, and aflatoxin B1 in various coffee bean samples, and to investigate the effectiveness of RF heating in eliminating fungal contamination. Additionally, the study assessed the rate of heating during RF treatment and quantified the levels of mycotoxins in RF-treated coffee beans.

Materials and methods

Survey of ochratoxin A and aflatoxin in different coffee beans by ELISA Test kit

A total of 98 samples at each coffee processing step was obtained randomly from warehouses in Northern Thailand, Southern Thailand, and Southern Laos. Preliminary assessment of these two types of mycotoxins was done using ELISA test kits (AgraQuant® Assay, Romer Labs Diagnostic GmbH).

Aflatoxin B1 analysis

Sample preparation. According to Romer Labs Aflatoxin B1 2/50 ELISA kit protocol (Romer Labs, 2019a), 5 g of the sample was weighted in a flask, 25 ml of 70 % methanol solution were added, and it was shaken for 3 minutes. After that the sample was allowed to settle, the top layer of extract was filtered through a Whatman #1 filter and the filtrates were collected. Then, dilution of the filtrate 1:2 with the assay buffer provided was carried out. The resultant solution was used for determinations.

Aflatoxin B1 content

An 8-channel pipette was used to dispense 200 µL of conjugate solution into each dilution well. Subsequently, 100 µL of each standard (i.e., 0, 2, 5, 20, and 50 ppb) or sample was added to the wells containing the conjugate. Immediately afterward, 100 µL from each dilution well was transferred to the corresponding antibody-coated microwell. The plate was incubated at room temperature for 15 minutes. After incubation, the wells were emptied and washed five times with distilled or deionized water. Then, 100 µL of substrate solution was added to each microwell, followed by a 5-minute incubation at room temperature. The reaction was stopped by adding 100 µL of stop solution to each well. A positive result was indicated by a color change from blue to yellow. Finally, the absorbance was measured at 450 nm using a microwell plate reader.

Ochratoxin analysis

Sample preparation. According to Romer Labs Ochratoxin 2/40 ELISA kit protocol (Romer Labs, 2019b), 5 g of green bean coffee was put in a flask, 25 ml of 70 % methanol solution was added, and it was shaken for 3 minutes. After that, the sample was allowed to settle, then the top layer of the extract was filtered through a Whatman #1 filter paper and the filtrate was collected. The resultant solution was used for determinations.

Ochratoxin content

An 8-channel pipette was used to dispense 200 µL of conjugate solution into each dilution well. Subsequently, 100 µL of each standard (0, 2, 5, 20, and 40 ppb) or sample was added to the wells containing the conjugate solution. Immediately after mixing, 100 µL from each dilution well was transferred into the corresponding antibody-coated microwell. The plate was then incubated at room temperature for 10 minutes. Following incubation, the wells were emptied and washed five times with either distilled or deionized water. Next, 100 µL of substrate solution was added to each microwell and the plate was incubated at room temperature for an additional 5 minutes. The reaction was terminated by adding 100 µL of stop solution to each well. A positive reaction was indicated by a color change from blue to yellow. Finally, the absorbance of each well was measured at 450 nm using a microwell plate reader.

Total aflatoxins analysis

Sample preparation. According to Romer Labs Total Aflatoxin 4/40 ELISA kit protocol (Romer Labs, 2019c). About 5 g of sample was weighted in a flask, 25 ml of 70 % methanol solution was added, and it was shaken for 3 minutes. After that, the sample was allowed to settle, then the top layer of the extract was filtered through a Whatman #1, the filtrate was collected and the resultant solution was used for determinations.

Total aflatoxin content

Using an 8-channel pipette, 200 µL of conjugate solution was dispensed into each dilution well. Then, 100 µL of each standard (i.e., 0, 4, 10, 20, and 40 ppb) or sample was added to the wells containing the conjugate. Immediately afterward, 100 µL from each dilution well was transferred to the corresponding antibody-coated microwell. The plate was incubated at room temperature for 15 minutes. Following incubation, the wells were emptied and washed five times with distilled or deionized water. Subsequently, 100 µL of substrate solution was added to each microwell, and the plate was incubated at room temperature for an additional 5 minutes. The reaction was terminated by adding 100 µL of stop solution to each microwell. A positive result was indicated by a color change from blue to yellow. Finally, the absorbance of the solution was measured at a wavelength of 450 nm using a microwell plate reader.

Radio frequency heating treatments

The green coffee beans (*Coffea arabica* L.) used in the experiment were obtained from a farm in Mae Hong Son, Northern Thailand with a moisture

content of 9%. Samples were divided by initial condition and were conditioned to 16% moisture content to help fungi grow by mixing with a calculated amount of distilled water. The initial and modified green coffee beans were inoculated with *Aspergillus ochraceus* and *Aspergillus parasiticus*. RF heating of coffee beans was carried out using a pilot scale, 27.12 MHz, 3,000 kW RF system (Institute of Agriculture Engineering, University of Gottingen, Germany). The inoculated seeds were kept for 1 day before being treated. A test was carried out on the efficacy of RF at 27.12 MHz with the temperature at 55.00 °C for 180 seconds; preliminarily beans were directly plated on water agar (WA) plates with 10 beans per plate. All the plates were incubated at 28.00 °C for 72 hours. Fungal contamination was observed. Each fungal colony that appeared on coffee beans was counted visually and the percentage of contamination was calculated.

Radio frequency treatment evaluation

Quantitative assessment was conducted after RF treatments. The ability of the RF treatments to decrease the levels of contamination in the samples of *Aspergillus ochraceus* and *Aspergillus parasiticus* inoculated coffee bean was assesed following preparation and extraction before quantification by ELISA test kits. The experiments were conducted according to the protocols of the supplier of the quantitative test kits for OTA and AFs, Romer Labs.: AgraQuant® Total Aflatoxin, AgraQuant® Ochratoxin and AgraQuant® Aflatoxin B1.

Results

Survey of ochratoxin A and aflatoxin in different coffee beans by ELISA Test Kit

A total of 98 coffee samples comprising dried cherry coffee, parchment coffee, and green coffee beans were randomly collected from warehouses located in Northern Thailand, Southern Thailand, and Southern Laos (Figure 1). Mycotoxin levels in these samples were determined using enzyme-linked immunosorbent assay (ELISA) test kits. The results of the analysis are presented for ochratoxin A, aflatoxin B1, and total aflatoxins. Among the 98 analyzed samples, 10 representative samples were selected from each region (Figure 2). The incidence and concentration of mycotoxins varied across sample types and regions (Table 1). Ochratoxin A contamination ranged from below the limit of quantification (not detected) to 46.40 µg/kg in Robusta coffee, and from not detected to 25.21 µg/kg in Arabica coffee. Although OTA was not detected in

some samples, several of these showed relatively high levels of aflatoxin B1 and total aflatoxins, which could pose significant health risks to consumers.

Figure 1. Coffee processing step: Dried cherry coffee (A); Parchment coffee (B); Green coffee beans (C)

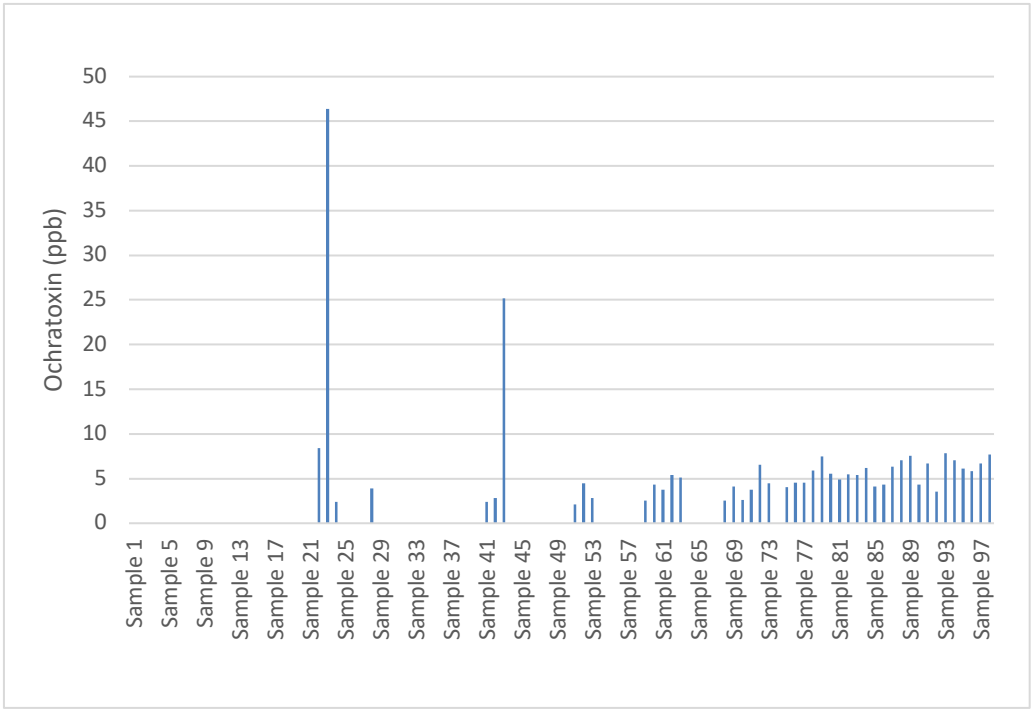

Figure 2. Level of contamination of ochratoxin detected in 98 samples of coffee beans by ELISA kits

Table 1. Level of contamination of ochratoxin A, aflatoxin B1 and total aflatoxins detected in coffee beans from Northern Thailand, Southern Thailand and Southern Laos

Coffee samples sites[1]	Species	Coffee sample type	Mean level of contamination		
			Ochratoxin (µg / kg)	Aflatoxin B1 (µg / kg)	Total Aflatoxins (µg / kg)
S1	Robusta	Dried Cherry Coffee	ND[2]	50.44	31.75
S2	Robusta	Parchment Coffee	ND	32.69	18.19
S3	Robusta	Green Coffee Beans	ND	29.67	20.69
S4	Robusta	Green Coffee Beans	46.40	18.73	10.18
N1	Arabica	Parchment Coffee	2.37	29.44	24.19
N2	Arabica	Defective Coffee	25.21	21.44	22.58
N3	Arabica	Green Coffee Beans	ND	32.34	19.79
N4	Arabica	Green Coffee Beans	ND	28.67	14.48
N5	Arabica	Green Coffee Beans	ND	31.13	15.57
L1	Arabica	Green Coffee Beans	ND	37.15	15.01

[1]/ Sites: S, Southern Thailand; N, Northern Thailand; L, Southern Laos
[2]/ ND: Not detected

Radio frequency heating treatments

An experiment was carried out to study an appropriate temperature and exposure time of RF for elimination of *Aspergillus* species inoculated to the green coffee beans (Figure 3). *Aspergillus ochraceus* and *Aspergillus parasiticus* were revived from arabica bean and re-identified by using Biolog FF microplate at Bacteria Plant Disease Laboratory Faculty of Agriculture, Chiang Mai University. The isolates of *Aspergillus* group were inoculated in potato dextrose agar (PDA) incubated at 28.00 °C for 72 hours. Two agar plugs were added in 400 grams arabica coffee beans. The inoculated seeds were kept for 24 hours before being treated. A test was carried out on the efficacy of RF at 27.12 MHz with the temperature at 55.00 °C for 180 seconds to eliminate the fungal contamination.

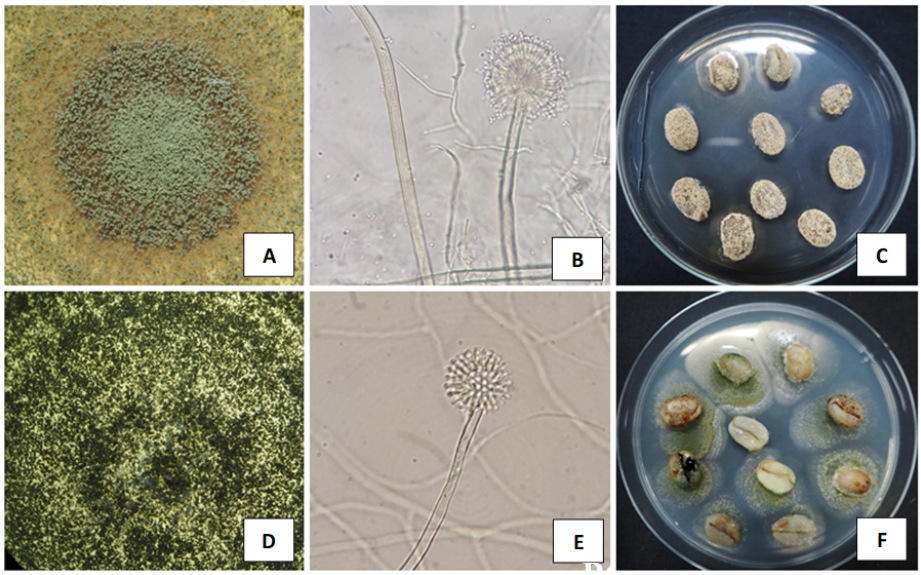

Figure 3. *Aspergillus* sp. isolated from coffee bean: *Apergillus ochraceus* colony on Potato dextrose agar under a stereo microscope (14 days) (A); hyphae conidia and conidiophores (B); fungal colonies formed on all inoculated coffee beans (C); *Apergillus parasiticus* colony on Potato dextrose agar under a stereo microscope (14 days) (D); hyphae conidia and conidiophores (E); fungal colonies formed on all inoculated coffee beans (F)

The results from preliminary culturing the inoculated coffee beans on WA (agar method) showed that the use of RF at the temperature of 55.00 °C for 180 seconds did not decrease the infection percentage of inoculated seeds with both of *A. ochraceus* and *A. parasiticus* (100 percent), same as the control treatment (non-RF treated).

Toxins quantitative assessment

The results showed the quantitative assessment of ochratoxin, aflatoxin B1 and total aflatoxin in green coffee beans inoculated with *A.ochraceus* and *A. Parasiticus* (Table 2) . After using RF at 55.00 °C for 180 seconds, it showed levels of contamination with ochratoxin and aflatoxin at 2.81 µg/kg and ND respectively, compared to inoculated coffee beans (16 % mc) which was 4.74 µg/kg and 2.75 µg/kg levels of contamination. The level of contamination was slightly decreased with RF treatment of coffee beans at 9 % mc, but RF treatment of coffee beans at 16 % moisture content increased the level of contamination when compared with the control. The incidence of aflatoxin B1 in coffee beans

inoculated wth *A. parasiticus* had a mean level of 65.33 µg/kg after RF treatment which was effective in decreasing the percentage of contamination when compared with the control. The RF at 55.00 °C for 180 seconds, did not decrease the percentage of contamination by OTA when compared with the control. Also, the moisture content was affected the incidence averages as the general averages showed a rise in the number of fungi and then decreased when moisture content of coffee bean is 9 %.

Table 2. Level of contamination of ochratoxin, aflatoxin B1 and total aflatoxin detected in coffee beans after being treated with a radio frequency of 27.12 MHz, at 55.00 °C, for 180 seconds as indicated by ELISA kits

Sample	Quality parameters	Moisture content (%)	Ochratoxin (µg / kg)	Aflatoxin B1 (µg / kg)	Total Aflatoxins (µg / kg)
Coffee bean	**Control**	9	ND[1/]	22.69	24.49
	RF	9	ND	20.82	24.57
	Control	16	2.90	29.19	25.73
	RF	16	3.05	29.07	22.46
Coffee bean inoculated *Aspergillus ochraceus*	**Control**	9	4.04	27.07	22.81
	RF	9	2.81	21.45	22.01
	Control	16	3.52	27.62	21.45
	RF	16	4.74	31.41	23.65
Coffee bean inoculated *Aspergillus parasiticus*	**Control**	9	ND	65.33	35.16
	RF	9	ND	39.55	27.13
	Control	16	2.09	87.47	46.37
	RF	16	2.75	148.48	55.61

1/: ND: Not detected

Discussion

Total aflatoxins and aflatoxin B1 were detected in 100% of the analyzed samples, with the highest concentration of 31.75 µg/kg observed in sample S1 (dried cherry coffee). Aflatoxin concentrations in both samples exceeded the maximum permissible limit of 4 µg/kg for total aflatoxins (B1, B2, G1, and G2) in food intended for direct human consumption, as established by the European Commission Regulation (2006). Although Noonim *et al*. (2005) reported that Robusta coffee is generally more contaminated with ochratoxin A (OTA) than Arabica, the present study found no correlation between coffee species and mycotoxin levels. Using Ridascreen® OTA ELISA kits, OTA contamination was observed in 98% of the samples, with levels ranging from <0.6 to 5.5 µg/kg in Arabica and 1 to 27 µg/kg in Robusta.

In this study, mycotoxin incidence appeared to be more strongly associated with post-harvest handling and storage conditions, particularly the location and

management of the warehouse. Cui *et al*. (2020) emphasized the need to optimize radio frequency (RF) heating parameters—such as frequency, grain type, and moisture content—to effectively control fungal contamination in stored grains. Similarly, Daou *et al*. (2021) demonstrated that environmental factors such as temperature, water activity, and relative humidity play a critical role in promoting fungal growth, aligning with findings from previous research (Al-Abdalall and Al-Talib, 2014; Bokhari, 2007).

In the current study, levels of ochratoxin A, aflatoxin B1, and total aflatoxins in green coffee bean samples were quantified using AgraQuant® ELISA kits. The most representative samples showed high levels of mycotoxin contamination. While OTA was detected at relatively low concentrations, total aflatoxins and aflatoxin B1 levels significantly exceeded regulatory limits, posing potential health risks and contamination hazards in coffee products. Moreover, RF heating treatments were found to be ineffective in eliminating mycotoxins from green coffee beans. The findings underscore the critical role of moisture content in influencing fungal contamination in stored coffee beans.

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