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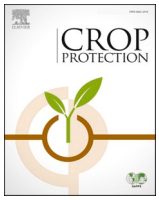

Circular economy approach to coffee processing residue valorization: Bioactive by-product extracts for managing pre- and post-harvest fungal pathogens

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ABSTRACT

The coffee industry generates a large amount of waste that is usually discarded, creating an environmental and economic problem. However, these by-products can be a valuable source of bioactive compounds with antimicrobial properties and present an opportunity for use in crop protection, either pre- or post-harvest. Following the principles of the circular economy, this study proposes the extraction and characterization of bioactive products from coffee by-products, as well as the evaluation of their antifungal activity against pathogens that affect coffee plants and/or stored coffee beans, such as *Fusarium xylarioides*, *Aspergillus flavus*, *A. niger*, and *Penicillium verrucosum*. *In vitro* activity assays demonstrate high antimicrobial activity of the husk, parchment, defective green beans with silverskin, and silverskin extracts, with minimum inhibitory concentrations ranging from 15.6 to 375 μg mL^{-1} against *F. xylarioides*, 31.2–1000 μg mL^{-1} against *A. flavus*, 62.5–1000 μg mL^{-1} against *A. niger*, and 62.5–1500 μg mL^{-1} against *P. verrucosum*, depending on the by-product extract used. The most effective extract, derived from silverskin, was evaluated for pre-harvest protection of coffee plants and demonstrated complete inhibition of *F. xylarioides*-induced tracheomycosis at 15.6 μg mL^{-1}. In turn, a concentration of 62.5 μg mL^{-1} of the silverskin extract was sufficient to prevent fungal growth of *A. flavus*, *A. niger*, and *P. verrucosum* on coffee beans. This concentration also prevented mycotoxin production by *A. flavus*, while a higher concentration of 125 μg mL^{-1} was required to prevent aflatoxin production by *A. niger*. The reported findings support coffee by-products extracts as promising alternatives to synthetic fungicides, with the potential to improve the sustainability of the coffee industry.

1. Introduction

Coffee, the world's most important food commodity and second most traded product after petroleum, is derived from tropical shrubs or small trees of the genus *Coffea* (family Rubiaceae), native to Africa and Asia. Currently cultivated in over 70 countries, global coffee production is predominantly concentrated in Brazil, Vietnam, and Colombia, which collectively account for more than 50 % of worldwide output (Esquivel and Jiménez, 2012; Serna-Jiménez et al., 2022). Among the 124 known *Coffea* species, commercial production is dominated by two varieties: Arabica (*Coffea arabica* L.), known for its superior organoleptic properties (sensory characteristics such as taste, aroma, texture, and appearance) and premium market prices, representing 75 % of global trade (Lee et al., 2023), and Robusta (*Coffea canephora* Pierre ex A. Froehner).

The coffee cherry (10−15 mm in length) consists of distinct anatomical layers encasing two coffee beans: an outer skin (exocarp), pulp (mesocarp), mucilage layer, parchment (endocarp), and silverskin

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(tegument) (Esquivel and Jiménez, 2012).

Coffee processing generates substantial waste, differing by processing method: dry processing yields approximately one ton of husks (combined exocarp, mesocarp, and parchment) per ton of coffee, while wet processing produces separate streams of pulp (43.2 % of fruit weight), mucilage (11.8 %), and parchment (6.1 %) (Esquivel and Jiménez, 2012). Secondary waste streams include harvest residues (plant leaves and flowers), spent coffee grounds, and rejected beans (immature, discolored, or damaged) (Cruz, 2014).

With annual coffee industry waste exceeding 10 million tons, representing over 50 % of coffee fruit biomass (Blinová et al., 2017; Lee et al., 2023), these by-products offer significant valorization opportunities. Industrial applications extend from energy production and compound adsorption to the extraction of high-value compounds including ethanol, gibberellic acid, α-amylase, and phenolic acids (chlorogenic, quinic, and ferulic acids). Furthermore, the abundance of phenolic antioxidants and phytonutrients makes these by-products particularly promising as functional ingredients in the agri-food industry (Esquivel and Jiménez, 2012), although their properties are species-dependent.

The antimicrobial properties of these by-products are of particular interest and position them as sustainable alternatives to synthetic phytosanitary products for crop protection, though efficacy varies according to coffee species and processing methods. Recent applications demonstrate this potential: coffee husks have been utilized in biopesticide production through fermentation with *Bacillus sphaericus* Meyer and Neide, 1904 and *Bacillus thuringiensis* serovar *israelensis* de Barjac, 1978; Lee et al. (2023). Additionally, hydroethanolic extracts from parchment have been incorporated as antifungal additives in gellan gum-based films for food preservation, showing effectiveness against *Fusarium* sp. and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Mirón-Mérida et al., 2019). Regarding silverskin, to the authors' knowledge, antimicrobial studies have been limited to human pathogens, notably *Staphylococcus*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Candida*, *Listeria,* and *Salmonella*, with inhibition values ranging from 31.5 to 75,000 μg mL^{-1}. This substantial variability is attributed to differences in extraction methods (solvent type, temperature, time), the variable susceptibility of target strains, and variations in bioactive compound composition according to coffee species, geographical origin, and processing methods (Nzekoue et al., 2020; Rodrigues et al., 2014; Ziemah et al., 2024). This by-product is also utilized in food formulations (Gemechu, 2020), composting, or as fuel (Esquivel and Jiménez, 2012), similar to spent coffee grounds (Gemechu, 2020; Lee et al., 2023), which exhibit antioxidant and antitumoral properties (Esquivel and Jiménez, 2012).

This research aims to extract and characterize bioactive products from various coffee by-products —namely husk, parchment, defective green beans with silverskin, and silverskin extracts—, and examine their antifungal activity through *in vitro*, *in planta*, and *ex-situ* trials against coffee plant pathogens, specifically *Fusarium xylarioides* Steyaert, the causative agent of coffee wilt disease affecting African *Coffea* species (Rutherford, 2006), and three potential mycotoxin-producing storage fungi —*Aspergillus flavus* Link (Commission, 2006), *Aspergillus niger* Tiegh. (García-Moraleja et al., 2015; Taniwaki et al., 2006), and *Penicillium verrucosum* Dierckx (Cabañes et al., 2010)—.

2. Materials and methods

2.1. Coffee by-products, plants and beans

Peruvian Arabica coffee by-products, representative of the dry processing method, were provided by Olam Agro Perú S.A.C. (Lima, Peru), a concessionaire of Olam International Limited (Olam Group, Singapore). The company supplied two separate sample batches: the first consisted of husk material (exocarp and part of the mesocarp) obtained through controlled drying, while the second comprised parchment coffee, i.e., coffee beans dried for no longer than two weeks with moisture content below 12 %. The parchment layer was manually separated and removed from the defective green coffee beans, which still retained their silverskin.

Due to the difficulties associated with manual silverskin separation, commercial silverskin was acquired from Perk Environmental Ltd. (Maple Ridge, British Columbia, Canada). This silverskin was obtained from the roasting and grinding process of organic Arabica coffee.

For *in planta* assays, *C. arabica* plants were supplied by IKEA (Valladolid, Spain) and grown under controlled greenhouse conditions. The plants were at least 80 days old at the time of the experiment, corresponding to the juvenile vegetative stage with 4–6 pairs of fully developed true leaves. Plants were maintained at 25 °C under a 16/8 h light/dark photoperiod throughout the experimental period. *Coffea arabica* typically begins flowering after 3–4 years under optimal conditions, but our experimental plants were in their first year and had not reached reproductive maturity. The plants exhibited characteristic elliptical, dark green leaves with prominent veins and were grown in 9 cm pots containing sterile peat substrate.

For *ex-situ* assays, Arabica coffee beans were provided by Seda Outspan Iberia S.L. (Palencia, Spain). Approximately 500 g of green coffee beans at commercial maturity were used, processed via standard wet processing methods and stored at 20 °C with 60 % relative humidity to maintain quality until experimental use.

2.2. Reagents and fungal isolates

Ammonium hydroxide, 50 % *v/v* aqueous solution (CAS No. 1336-21-6), was purchased from Alfa Aesar (Ward Hill, MA, USA). Acetic acid (80 % in H_2O; CAS No. 64-19-7) was supplied by Sigma Aldrich Química S.A. (Madrid, Spain), while Becton, Dickinson, and Co. (Franklin Lakes, NJ, USA) provided potato dextrose broth (PDB) and potato dextrose agar (PDA).

Fungal isolates of *A. flavus* (MYC-1629, isolation source: contaminated cheese rind) and *A. niger* (MYC-1600, isolation source: almond shell) were supplied as PDA subcultures by the Agrifood Research and Technology Centre of Aragón (CITA; Zaragoza, Spain), while *F. xylarioides* (DSM No. 62457; isolation source: *Coffea excelsa*) and *P. verrucosum* (DSM No. 1836; isolation source: not provided) were acquired from the Leibniz Institute DSMZ collection (Braunschweig, Germany).

For comparative assessment of antifungal efficacy, three commercial synthetic fungicides were employed as positive controls: Ortiva® (azoxystrobin 25 %; Syngenta, Basel, Switzerland), Vondozeb® (mancozeb 75 %; UPL Iberia, Barcelona, Spain), and Fesil® (fosetyl-Al 80 %; Bayer, Leverkusen, Germany). These fungicides were supplied by the Plant Health and Certification Center (CSCV) of the Gobierno de Aragón (Zaragoza, Spain). The fungicides were tested at two concentrations: the manufacturer's recommended dose and one-tenth of the recommended dose.

2.3. Extraction process

The extraction process followed the methodology previously described by Sánchez-Hernández et al. (2022b). A probe-type ultrasonicator (model UIP1000hdT; 1000 W, 20 kHz; Hielscher Ultrasonics, Teltow, Germany) was used to sonicate the by-product samples for 10 min in pulsed mode, with a 2-min pause after each 2.5 min of sonication, followed by a 24-h resting period. Subsequently, acetic acid was used to adjust the pH to neutral. After centrifuging the solution at 9000 rpm for 15 min, the supernatant was filtered using Whatman No. 1 paper. Extract lyophilization was performed using a LyoQuest-55 Plus freeze-dryer (Azbil Telstar SLU, Barcelona, Spain).

2.4. Characterization of by-products and their extracts

The infrared (IR) spectra of the by-products before extraction and of the freeze-dried extracts were recorded using a Thermo Scientific

Nicolet iS50 Fourier-transform infrared (FTIR) spectrometer (Waltham, MA, USA), equipped with an attenuated total reflection (ATR) system. Spectra were recorded in the 400−4000 cm^{-1} range with 1 cm^{-1} spectral resolution, averaging 64 scans.

The aqueous ammonia extracts (neutralized with acetic acid) of the by-products were analyzed by gas chromatography-mass spectrometry (GC−MS) at the Research Support Services (STI) of the University of Alicante (Alicante, Spain), using an Agilent Technologies (Santa Clara, CA, USA) 7890A gas chromatograph coupled to a 5975C quadrupole mass spectrometer. Chromatographic conditions were as follows: injection volume = 1 μL; injector temperature = 280 °C, in splitless mode; initial oven temperature = 60 °C for 2 min, followed by a 10 °C·min^{-1} ramp to a final temperature of 300 °C for 15 min. Compound separation was performed using an Agilent Technologies HP-5MS UI column (30 m length, 0.250 mm diameter, 0.25 μm film). Mass spectrometer conditions: electron impact source temperature = 230 °C, quadrupole temperature = 150 °C; ionization energy = 70 eV. Component identification was based on a comparison of mass spectra and retention times with authentic compounds and computerized matching with the National Institute of Standards and Technology database (NIST11).

2.5. In vitro antifungal activity assessment

The antifungal activity of coffee by-product extracts and commercial synthetic fungicides was evaluated following the EUCAST standard method (Arendrup et al., 2012). By-product extract solutions were incorporated into the PDA medium to achieve concentrations ranging from 3.9 to 1500 μg mL^{-1}. Fungal mycelium plugs from one-week-old PDA cultures of *F. xylarioides*, *A. flavus*, and *A. niger*, and from two-week-old cultures of *P. verrucosum*, were transferred to plates containing specified concentrations for each treatment. Three plates per treatment/concentration combination were prepared. The entire antifungal susceptibility experiment was conducted twice independently. Incubation conditions were specific to each fungus: *F. xylarioides*, *A. flavus*, and *A. niger* plates were incubated at 25 °C in darkness for one week, and *P. verrucosum* for two weeks. The untreated control consisted of non-amended PDA medium. Radial mycelial growth was evaluated as the mean of two perpendicular colony diameters in each replicate. Growth inhibition was determined using the formula $[(d_c - d_t)/d_c] \times 100$, where d_c and d_t represent the mean diameter of untreated control and treated fungal colonies, respectively. Each treatment was performed in triplicate (three plates per treatment/concentration combination) and the entire experiment was conducted twice independently. Results are presented as mean values from all replicates. The minimum inhibitory concentration (MIC)—the lowest concentration that completely inhibits visible fungal growth compared to the untreated control, determined by visual assessment and measurement of colony diameter—was determined as the lowest concentration showing complete inhibition of visible fungal growth in all replicates.

2.6. In planta protection assays

Coffea arabica plants (≥80 days old) were used in the *in planta* experiments to evaluate the efficacy of the most active *in vitro* treatment (coffee silverskin extract) against artificially inoculated *F. xylarioides*, following Sánchez-Hernández et al. (2023b) and González et al. (2020) with minor modifications. The plants were grown in 9-cm pots containing sterile peat substrate (15 plants per treatment, two independent repetitions). For the silverskin-extract treatment, roots were first immersed for 2 min in the extract solution (15.6 μg mL^{-1}), then allowed to air-dry until surface moisture was no longer visible, and immediately thereafter immersed for 2 min in a 1×10^6 conidia·mL^{-1} suspension of *F. xylarioides* (prepared as in Sánchez-Hernández et al. (2023a)). Positive controls were treated identically but with roots immersed in sterile water instead of extract before the conidial inoculation step. Negative controls consisted of roots immersed in sterile water for 2 min, air-dried, and then re-immersed in sterile water for 2 min. Prior to inoculation, the fungus was cultured in 250 mL flasks of PDB for 7 days at 25 °C in darkness with constant agitation. All pots were maintained in a growth chamber at 25 °C under a 16/8 h light/dark photoperiod for 30 days. The experiment was conducted twice.

2.7. Coffee bean protection assays and mycotoxin determination

For fungal conidial suspension preparation, conidia were obtained from one-week-old PDB cultures of *F. xylarioides*, *A. flavus*, and *A. niger*, and two-week-old PDB cultures of *P. verrucosum*. After filtration through two layers of sterile muslin to remove somatic mycelia, spore concentration was determined using a hemocytometer (Weber Scientific International Ltd., Teddington, Middlesex, UK) and adjusted to 1×10^6 conidia·mL^{-1}.

The effect of silverskin by-product extract on coffee bean protection against *A. flavus*, *A. niger*, and *P. verrucosum* was determined according to Sánchez-Hernández et al. (2023a), with slight modifications. Beans were surface-sterilized by immersion in 3 % sodium hypochlorite for 2 min and then rinsed with sterile milli-Q water three times before drying at room temperature in a laminar flow hood on sterile absorbent paper. Bean treatments (50 g of coffee beans per treatment) were conducted by immersion in 100 mL of silverskin extract (at a concentration of either 62.5 or 125 μg mL^{-1}, adding 0.2 % Tween® 20) at room temperature, under agitation, for 15 min. Positive and negative controls used distilled water with 0.2 % Tween® 20. After drying for 30 min at room temperature in a laminar flow hood, beans were inoculated with the above-mentioned conidial suspension. Subsequently, samples were incubated in a dark chamber at 25 °C for 28 days. Each treatment was repeated three times, and the experiment was repeated twice.

To evaluate whether the silverskin treatment, in addition to inhibiting pathogen growth on the coffee beans, also prevented mycotoxin production, an analytical method was implemented for the detection and quantification of total aflatoxins (AFT) —B_1, B_2, G_1, and G_2— and ochratoxin A (OTA). Two separate 5−10 g subsamples of dried coffee beans were extracted: one with 20 mL of methanol:water (80:20 *v/v*) for AFT analysis and another with 20 mL of methanol:sodium bicarbonate 3 % (50:50 *v/v*) for OTA analysis. Samples were homogenized by vortexing using a TK3S test tube shaker (Techno Kartell, Noviglio, Italy) for 3–5 min. The resulting extracts were filtered through Whatman No. 4 filter paper (Symta, Madrid, Spain), then diluted with phosphate-buffered saline (PBS) solution and subjected to purification using AflaTest WB SR or OchraTest WB immunoaffinity columns (Vicam, Milford, MA, USA), following the manufacturer's instructions. The purified extracts were dried under N_2 stream at 50 °C in a sample concentrator (Stuart Instruments, Cambridge, UK) and reconstituted in 0.5 mL of mobile phase consisting of water/acetonitrile/methanol (50:10:40 *v/v/v*) for AFT or water/acetonitrile/acetic acid (51:48:1 *v/v/v*) for OTA. Finally, they were filtered through 0.45 μm membranes (Análisis Vínicos, Ciudad Real, Spain) immediately before injection into the chromatographic system.

The analysis was performed using an Agilent Technologies 1100 high-performance liquid chromatography (HPLC) system coupled to a fluorescence detector (FLD). Chromatographic separation was carried out on an Ace 5 C_{18} column (250 × 4.6 mm, 5 μm particle size; Análisis Vínicos, Ciudad Real, Spain) maintained at 25 °C. A manual injection system equipped with a 100 μL loop and a 250 μL syringe was used. The aforementioned mobile phases were pumped isocratically at a flow rate of 1 mL min^{-1}. For AFT detection, fluorescence parameters were set at 365 nm (excitation) and 435 nm (emission), while for OTA, they were set at 333 nm (excitation) and 460 nm (emission). The chromatographic system incorporated a photochemical reactor for enhanced detection (PHRED) (LCTech UVE, Dorfen, Germany) configured at 254 nm for post-column derivatization of aflatoxins.

The presence/absence of mycotoxins was determined using OpenLAB CDS v.2013 software (Agilent). The presence of mycotoxins was

considered positive when detected levels exceeded the established limits of detection (LOD—the lowest concentration of an analyte that can be reliably detected but not necessarily quantified): 0.02 μg kg^{-1} for each aflatoxin and 0.1 μg kg^{-1} for OTA. The limits of quantification (LOQ—the lowest concentration of an analyte that can be quantified with acceptable precision and accuracy) were 0.06 μg kg^{-1} for each aflatoxin and 0.5 μg kg^{-1} for OTA. Currently, Commission Regulation (EU) 2023/915 does not establish maximum levels for aflatoxins in coffee, despite being identified by the International Agency for Research on Cancer (IARC) as some of the most toxic mycotoxins; whereas for OTA, the limit is set at 3 μg kg^{-1} in roasted coffee, with maximum limits not yet established for unroasted coffee.

3. Results

3.1. Vibrational characterization of coffee by-products

Infrared spectra of coffee by-product residues and their extracts (Table 1) revealed characteristic changes during the extraction and freeze-drying processes, showing the disappearance of bands corresponding to water (ca. 3300 cm^{-1}) and chlorogenic acids (1730, 1517, 1370, 1316, 1146, and 1097 cm^{-1}). Simultaneously, other bands emerged: an ammonia contamination band (921–954 cm^{-1}) and, specifically in silverskin samples, a band at 1069 cm^{-1} attributed to quinic acid.

3.2. GC-MS characterization of coffee by-product extracts

The compounds identified in the aqueous ammonia extracts of the different coffee by-products are detailed in Tables S1–S4. The husk extract (Table S1) primarily contained methoxy-phenyl-oxime (41.03 %), caffeine (23.20 %), *N*-benzo[1,2,5]thiadiazol-5-yl-acetamide (7.12 %), and acetamide (6.18 %). The parchment extract (Table S2) predominantly featured methoxy-phenyl-oxime (49.60 %), caffeine (16.31 %), acetamide (9.04 %), diethyl phthalate (2.61 %), and 7*H*-dibenzo(*a, g*)carbazole (2.51 %). The defective green coffee beans with silverskin extract (Table S3) revealed caffeine (51.69 %), 3-(acetylamino)-benzoic acid methyl ester (11.69 %), methoxy-phenyl-oxime (5.66 %), acetamide (4.52 %), and *n*-hexadecanoic acid (4.13 %) as major constituents. The silverskin extract (Table S4) exhibited the most diverse chemical profile, primarily consisting of caffeine (18.49 %), acetamide (15.53 %), *N,N*-dimethylaminoethanol (8.53 %), *n*-hexadecanoic acid (6.36 %), quinic acid (1.84 %), octadecanoic acid (1.54 %), catechol (1.49 %), (*S*)-3-(1-methyl-2-pyrrolidinyl)-pyridine (1.43 %), and eicosanoic acid (1.42 %).

3.3. Antifungal activity of coffee by-product extracts

3.3.1. In vitro activity

The MICs from the antifungal susceptibility test for each coffee by-product are presented in Table 2. Silverskin extract demonstrated the highest antifungal efficacy among all tested by-products, with MICs of 15.6 μg mL^{-1} against *F. xylarioides*, 31.2 μg mL^{-1} against *A. flavus,* and 62.5 μg mL^{-1} against both *A. niger* and *P. verrucosum*. Conversely, the coffee husk extract exhibited the lowest antifungal activity, with inhibition values ranging from 375 to 1500 μg mL^{-1}. Results were consistent between the two independent experimental runs.

Comparative evaluation of commercial synthetic fungicides revealed variable efficacy patterns across pathogens and treatments (Table 3). Mancozeb demonstrated complete inhibition of *A. flavus*, *A. niger*, and *F. xylarioides* even at one-tenth of the recommended dose (150 μg mL^{-1}), though *P. verrucosum* showed 82.3 % inhibition at this concentration, requiring the full recommended dose (1.5 mg mL^{-1}) for complete

Table 2
Minimum inhibitory concentration values (in μg·mL^{-1}) against *F. xylarioides*, *A. flavus*, *A. niger*, and *P. verrucosum* obtained for each coffee by-product.

By-product	*F. xylarioides*	*A. flavus*	*A. niger*	*P. verrucosum*
Husk	375	1000	1000	1500
Parchment	250	750	750	1000
Defective green beans + silverskin	125	250	375	500
Silverskin	15.6	31.2	62.5	62.5

Table 1
Main FTIR spectral bands of the coffee by-product residues and their freeze-dried extracts, along with their assignments. Wavenumber values are given in cm^{-1}.

Husk + pulp	Husk + pulp Extract	Parchment	Parchment Extract	Bean + Silverskin	Bean + Silverskin Extract	Silverskin	Silverskin Extract	Assignment
–	–	3330	–	3293	–	3332	–	O–H stretching, hydrogen bonding
3306	3207	–	3202	–	3220	–	3201	O–H stretching, hydrogen bonding
2920	2926	2924	2930	2922	–	2918	2917	C–H stretching (asymmetric). In caffeine: 2922 cm^{-1}
2851	–			2851	2851	2850	2850	C–H stretching (symmetric). In caffeine: 2855 cm^{-1}
1730	–	1726	–	1730	1694	1727	–	Esters (characteristic of chlorogenic acid)
1620	1641	1614	1659	1605	1642	1631	1650	Esters (characteristic of chlorogenic acid)
1551	1579	–	1556	1551	1581	1547	1585	amide II (characteristic of plant proteins; acetamide)
1517	–	1518	–	1511	–	1513	–	Characteristic of chlorogenic acid
1440	1402	1425	1402	1422	–	1415	1401	O–H angular deformation of quinic acid/ chlorogenic acid
1372	–	1371	–	1370	1378	1370	–	Chlorogenic acid esters
1316	–	1317	–	1320	–	1316	–	O–H bending (characteristic of chlorogenic acid)
1238	1241	1240	1242	1233	1241	1233	1240	C–O and C–C stretching in carbohydrates (sucrose)
1146	–	1146	–	1143	–	1147	–	Characteristic of esters (chlorogenic acid)
1093	–	1096	–	1095	1100	1097	–	Characteristic of carbohydrates and chlorogenic acids
–	–	–	–	–	–	–	1069	C–O axial deformation of quinic acid
1016	1026	1013	1028	1018	1029	1022	1018	C–O and C–C stretching in carbohydrates (arabinogalactans)
–	921	–	924	–	922	–	954	C–C stretching. Characteristic of ammonia
–	–	893	867	895	–	897	892	Asymmetric deformation of cellulose and hemicellulose

Table 3
Radial growth of mycelium and inhibition percentages for commercial synthetic fungicides.

Commercial fungicide	Pathogen	Growth of mycelium (mm)			Inhibition (%)	
		Control (PDA)	Rd/10	Rd	Rd/10	Rd
Azoxystrobin	*A. flavus*	75.0	61.4	0.0	18.1	100.0
	A. niger	75.0	75.0	62.3	0.0	16.9
	F. xylarioides	75.0	21.7	11.7	71.1	84.4
	P. verrucosum	75.0	41.7	28.3	44.4	62.3
Mancozeb	*A. flavus*	75.0	0.0	0.0	100.0	100.0
	A. niger	75.0	0.0	0.0	100.0	100.0
	F. xylarioides	75.0	0.0	0.0	100.0	100.0
	P. verrucosum	75.0	13.3	0.0	82.3	100.0
Fosetyl-Al	*A. flavus*	75.0	50.0	20.0	33.3	73.3
	A. niger	75.0	75.0	58.3	0.0	22.3
	F. xylarioides	75.0	60.0	8.3	20.0	88.9
	P. verrucosum	75.0	58.9	45.5	21.5	39.3

control. Azoxystrobin achieved complete inhibition only against *A. flavus* at the recommended dose (62.5 mg mL^{-1}), with incomplete inhibition of other pathogens (*F. xylarioides*: 84.4 %, *P. verrucosum*: 62.3 %, *A. niger*: 16.9 %). Fosetyl-Al showed moderate activity, with highest inhibition against *F. xylarioides* (88.9 %) and *A. flavus* (73.3 %) at the recommended dose (2 mg mL^{-1}), but did not achieve complete inhibition against any pathogen tested.

Rd stands for the recommended dose, i.e., 62.5 mg mL^{-1} of azoxystrobin (250 g L^{-1} for Ortiva®, azoxystrobin 25 %), 1.5 mg mL^{-1} of mancozeb (2 g L^{-1} for Vondozeb®, mancozeb 75 %), and 2 mg mL^{-1} of fosetyl-Al (2.5 g L^{-1} for Fosbel®, fosetyl-Al 80 %). All mycelial growth values are average values ($n = 6$).

Importantly, the silverskin extract achieved complete inhibition at concentrations of 15.6–62.5 μg mL^{-1}, which are lower than both the recommended doses and one-tenth recommended doses of the commercial fungicides tested, suggesting potential for development as an efficient natural alternative.

3.3.2. *In planta assays*

Since silverskin extract showed the highest efficacy in the *in vitro* tests, it was selected for evaluating protection against *F. xylarioides* in coffee plants (Fig. 1). All positive control plants (artificially inoculated and treated only with bi-distilled water, double-distilled with purity >99.9 % and conductivity <1 μS cm^{-1}) exhibited typical symptoms of coffee wilt disease caused by *F. xylarioides*, such as root rot or necrosis of the taproot and basal stem portion, leading to easy uprooting. As the disease progressed, leaves blackened, curled, and wilted, ultimately leading to the collapse of the entire plant (Fig. 1b). In contrast, plants treated with silverskin extract (Fig. 1c) at a concentration of 15.6 μg mL^{-1} showed no symptoms of wilting or root rot and displayed no signs of phytotoxicity, appearing similar to the negative control plants (Fig. 1a). Results were consistent across both experimental repetitions, with 100 % protection observed in both trials.

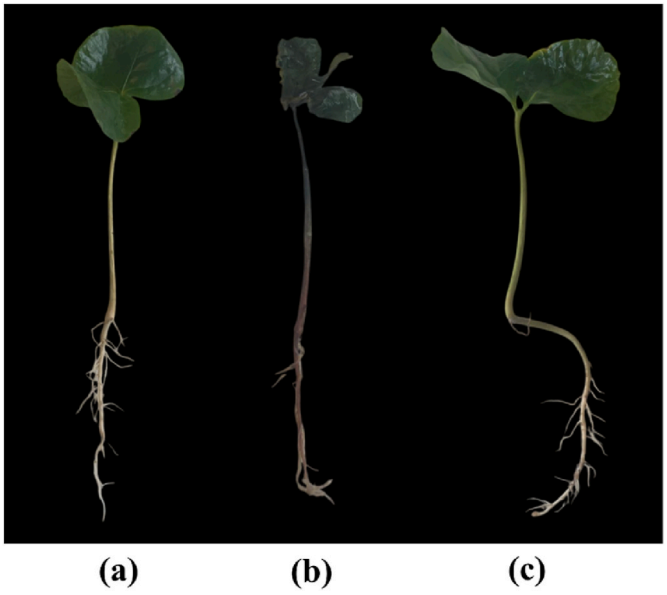

Fig. 1. Symptoms of coffee tracheomycosis caused by *F. xylarioides* on *C. arabica* plants after 30 days of inoculation: (**a**) negative control; (**b**) positive control; (**c**) silverskin extract at 15.6 μg mL^{-1}.

3.3.3. *Coffee bean postharvest protection*

To evaluate the efficacy of silverskin extract to provide postharvest protection of coffee beans, favoring their storability and food safety, *ex-situ* tests were carried out against *A. niger* (Fig. 2a and d), *A. flavus* (Fig. 2b and e), and *P. verrucosum* (Fig. 2c and f). After 28 days of incubation, in coffee bean samples artificially infected with each pathogen, no mycelial development was observed for those treated with silverskin extract at a concentration of 62.5 μg mL^{-1}, while positive control beans (inoculated and treated only with distilled water) exhibited profuse fungal colonization (Fig. 3). Results were reproducible across both experimental repetitions, demonstrating the reliability and consistency of the protective effect. Thus, the treatment showed a clear protective effect on stored coffee beans artificially exposed to the pathogens.

Mycotoxin analysis of coffee bean samples (Table 4) confirmed the absence of contamination in the negative control (untreated green coffee beans without pathogen), validating the quality and suitability of the experimental samples. The positive controls (green beans treated with

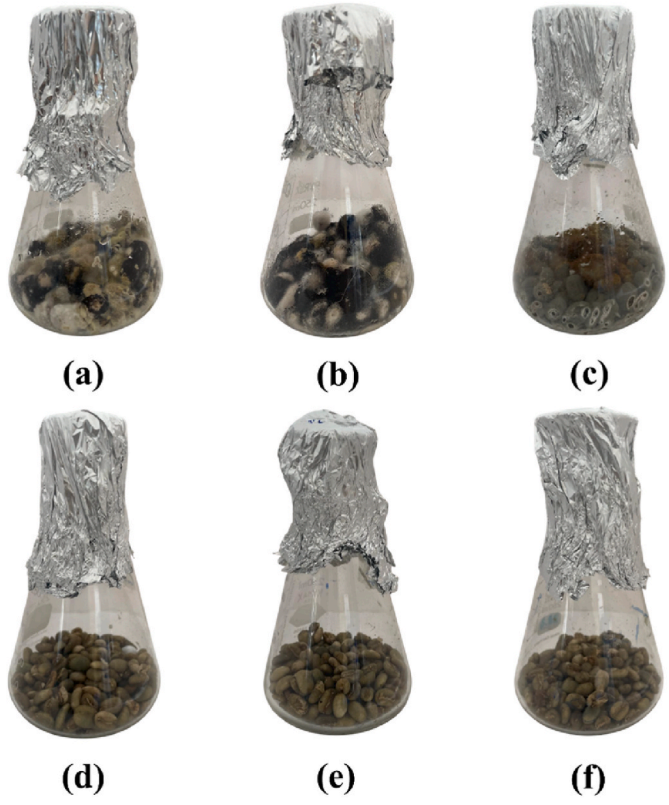

Fig. 2. Postharvest protection assay. Untreated coffee beans artificially inoculated with (**a**) *A. niger*, (**b**) *A. flavus*, and (**c**) *P. verrucosum*. Effect of applying silverskin extract at a concentration of 62.5 μg mL^{-1} to coffee beans on the growth of (**d**) *A. niger*, (**e**) *A. flavus*, and (**f**) *P. verrucosum*. Only one replicate per treatment is shown.

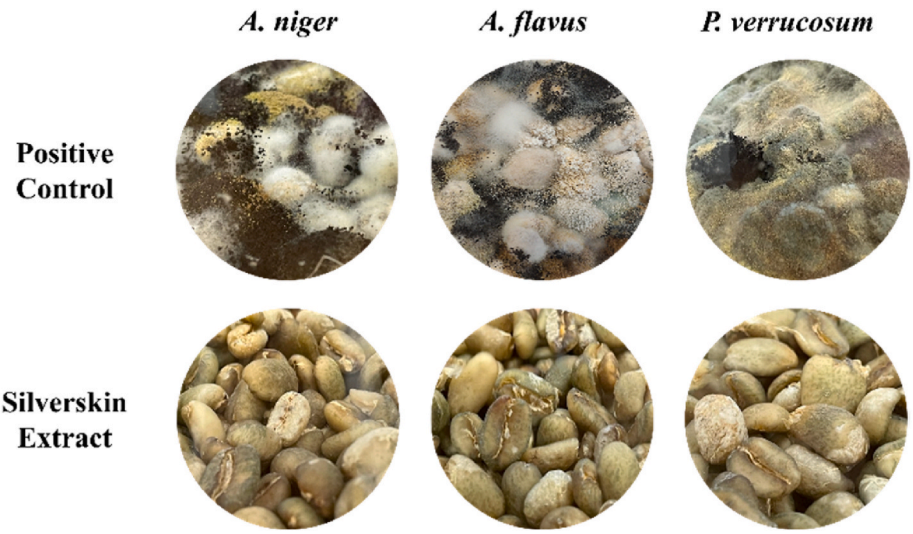

Fig. 3. Detail of coffee beans inoculated with *A. niger*, *A. flavus*, and *P. verrucosum*: (*top*) untreated; (*bottom*) treated with silverskin extract at a concentration of 62.5 μg mL^{-1}. Only one replicate per treatment is shown.

Table 4
Presence/absence of mycotoxins (total aflatoxins or ochratoxin) in coffee bean samples.

Pathogen	Treatment	AFT	OTA
Negative control		Negative	Negative
A. flavus	Positive control	Positive	Positive
	MIC	Negative	Negative
A. niger	Positive control	Positive	Positive
	MIC	Positive	Negative
	MIC × 2	Negative	Negative
P. verrucosum	Positive control	Negative	Negative
	MIC	Negative	Negative

AFT: Total of aflatoxins ($B_1 + B_2 + G_1 + G_2$); OTA: Ochratoxin A.

sterile water and artificially inoculated with pathogens) showed mycotoxin contamination for both *Aspergillus* species: *A. flavus* produced AFT and OTA at 0.24 and 0.15 μg kg^{-1}, respectively. Although *A. niger* is not typically recognized as an aflatoxin-producing species, some reports have suggested that atypical strains under certain environmental and culture conditions may produce trace amounts of aflatoxins (Al-Abdalall, 2009). This may explain the low levels detected in this study (0.02 and 0.1 μg kg^{-1} for AFT and OTA respectively). In samples treated with silverskin extract, a concentration of 62.5 μg mL^{-1} effectively prevented mycotoxin production by *A. flavus*. However, for *A. niger,* a higher concentration (125 μg mL^{-1}) was required to achieve values below the LOD, as AFT was detected at 0.04 μg kg^{-1} when using 62.5 μg mL^{-1}. No mycotoxins were detected in the *P. verrucosum* treatments, suggesting that the tested strain may not be mycotoxigenic under the experimental conditions used in this study.

4. Discussion

4.1. Chemical composition of the coffee by-products

Analysis of the chemical composition of the studied coffee by-product extracts revealed that caffeine, acetamide, and *n*-hexadecanoic acid were consistently present across all samples, with methoxy-phenyloxime serving as an additional major constituent in husk and parchment extracts, while quinic acid emerged as a component unique to silverskin extract. The relative abundance patterns varied among the different by-products. While methoxy-phenyloxime dominated both husk and parchment extracts (41.03 % and 49.60 %, respectively), caffeine became the predominant component in defective green beans with silverskin extract. Silverskin extract exhibited the most diverse chemical profile, containing the broadest range of identified compounds compared to the other by-products evaluated.

The high caffeine content in the extract of defective green coffee beans (52 %), along with the substantial levels in the extracts of husk (23 %), parchment (16 %), and silverskin (18.5 %) validate the proposed extraction procedure as potentially profitable for coffee by-product recovery, especially considering that caffeine prices have reached 133 €·kg^{-1}. Furthermore, the caffeine richness obtained when working in aqueous ammonia medium follows the same sequence reported by Machado et al. (2023), who employed hydromethanolic extraction: defective beans (1.4 %) > pulp (0.85 %) > silverskin (0.71 %) > parchment (0.06 %). Our caffeine contents were also higher than those compiled in the review by Lee et al. (2023), though the original sources cited therein do not specify extraction conditions: green coffee beans (0.8–1.4 % for Arabica, 1.7–4.0 % for Robusta), pulp from wet processing typically of Arabica (0.8–5.7 %), husk from dry processing typically of Robusta (0.5–2.0 %), and silverskin from roasting of both varieties (0.02–1.4 %). Similarly, Rebollo-Hernanz et al. (2023) reported wide ranges without specifying extraction parameters: husk from dry processing (6.8−12 %), pulp from wet processing (2−10.1 %), parchment from wet processing (0.1−1.3 %), and silverskin from both processing methods (0.7–9.5 %).

These substantial variations in reported caffeine content are multifactorial, reflecting both intrinsic factors—coffee species, by-product type, processing method (Arabica is usually wet-processed yielding pulp and parchment, while Robusta is typically dry-processed yielding husk), roasting degree, and fermentation conditions—and extrinsic analytical factors including extraction methodology (conventional solvent extraction, ultrasound-assisted, microwave-assisted, or supercritical fluid extraction), solvent polarity, temperature, time, solid-to-solvent ratio, and downstream purification steps. This complexity underscores the importance of standardized extraction protocols for meaningful comparisons between studies.

The presence of methoxy-phenyloxime in the extracts (a component typically associated with coffee's aroma and flavor profile) should be related to the temperature and duration of heating during processing. In the case of acetamide (a component identified in both roasted and instant coffee), its ubiquitous presence in the extracts must be attributed to the ammonolysis of acetate esters. Similarly, *n*-hexadecanoic acid could be related to chemical changes occurring during the extraction process.

The enrichment in quinic acid (associated with the bitterness and

astringency of reheated coffee) observed in silverskin extract may be due to either overexposure to heat of chlorogenic acid or its isomers (i.e., caffeoylquinic acids) or its hydrolysis in the aqueous ammonia medium, where the ester bond linking quinic acid to phenolic acids (such as caffeic or ferulic acid) is cleaved under alkaline conditions (Fig. 4), similar to that observed for defective green coffee beans, with the presence of methyl 3-(4-hydroxy-3-methoxyphenyl)-2-propenoate as a breakdown product.

Despite its high market price (between 1120 €·kg^{-1} and 2640 €·kg^{-1}) and its presence in silverskin (1.85 %) at levels comparable to the caffeine content in coffee beans (1.5 % in Arabica coffee and 2.5 % in Robusta coffee), the fact that this film is the smallest residual fraction (making its separation from other fractions tedious) confers only moderate expectations for its potential recovery. For comparative purposes, according to Machado et al. (2023), the presence of caffeoylquinic acids in silverskin hydromethanolic extracts was much lower (79.7 mg/100 g, or 0.08 %), reaching 4.88 % for defective beans (which had the highest content).

The absence of melanoidins in the GC-MS identification of compounds from silverskin extract should be attributed to their complexity and high molecular weight, as they are typically too large and insufficiently volatile for this technique. However, compounds structurally analogous to melanoidins or potential melanoidin fragments were observed, including nitrogen-containing compounds, furans, pyrroles, phenolic compounds, and carbonyl compounds. From the compounds list, several are potentially related or structurally analogous to melanoidins: *N,N*-dimethylaminoethanol, (*S*)-3-(1-methyl-2-pyrrolidinyl)-pyridine, and 2(1*H*)-pyridinone as carbon-nitrogen compounds; phenolic compounds catechol and vanillin, susceptible to incorporation into melanoidin structures; the cyclic ketone 2-hydroxy-2-cyclopenten-1-one and cyclic diketone 3-methyl-1,2-cyclopentanedione, potentially involved in melanoidin formation; the heterocyclic compound 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one as a melanoidin fragment; the nitrogenous heterocyclic compound 1*H*-pyrrole-2-carboxaldehyde, similar to melanoidins; and 5-hydroxymethylfurfural, a furanic derivative associated with the Maillard reaction and melanoidin formation. The presence of these compounds, though not conclusive, suggests that the silverskin extract contains Maillard reaction products, consistent with the presence of melanoidins in the original sample.

4.2. Antifungal activity

4.2.1. Presence of constituents with antifungal activity

Using the most effective extract (silverskin) as reference and considering only compounds with abundances greater than 0.8 %, the observed antifungal activity should be primarily attributed to the presence of caffeine, with antifungal and antimicrobial properties (Sánchez-Hernández et al., 2022a); *n*-hexadecanoic acid (palmitic acid) and its methyl ester, known for their antimicrobial activity (Ganesan et al., 2024; Patel et al., 2024); quinic acid, which demonstrates antifungal and antimicrobial activity (Heena et al., 2024; Muthamil et al., 2018); octadecanoic acid (stearic acid), which possesses antimicrobial properties (Guimarães and Venâncio, 2022); catechol, which shows both antibacterial and antifungal effects (Kocaçalışkan et al., 2006); eicosanoic acid (arachidic acid), which shows antifungal activity (Pereira et al., 2016); *β*-sitosterol, with both antifungal and antibacterial properties (Kiprono et al., 2000); oleic acid, which exhibits antimicrobial properties (Guimarães and Venâncio, 2022); and vanillin, with antifungal activity (Li and Zhu, 2021).

4.2.2. Mechanism of action

Given that caffeine is the most abundant component among those previously mentioned, it is pertinent to discuss its mechanism of action as an antifungal agent. Methylxanthines in general present interesting antifungal activity, as they inhibit fungal chitinases necessary for fungal cell wall remodeling and cell replication (Tsirilakis et al., 2012), and several studies have proposed caffeine as an ecological, safe, and affordable alternative to conventional biocides for wood protection (Kobetičová et al., 2020; Pánek et al., 2021; Šimůnková et al., 2021).

Regarding its mode of action, caffeine is known to act against fungi in two ways: first, by directly suppressing growth and, second, by promoting mycoparasitism of hyperparasitic species (Sugiyama et al., 2016). Concerning the first mechanism, Reinke et al. (2006) reported that the fungicidal action of caffeine was based on damaging the yeast cytoplasmic membrane during the early stages of formation. Wang et al. (2016) analyzed *in vitro* the antifungal mechanisms of caffeine against *Colletotrichum fructicola* Prihastuti, L. Cai & K.D. Hyde, studying its effects on mycelium morphology, cell wall, and plasma membrane. They discovered that caffeine induced oxidative stress in fungal cells, evidenced by significantly increased malondialdehyde (MDA) levels—a biomarker of lipid peroxidation that indicates membrane damage—and altered superoxide dismutase (SOD) activity, reflecting the cellular antioxidant response to reactive oxygen species (ROS) generation. These oxidative stress markers correlated with observable morphological changes including cell wall thickening, organelle degradation, and plasma membrane disruption, confirming that caffeine exerts its antifungal effects through ROS-mediated cellular damage that ultimately leads to growth inhibition or pathogen death.

Based on the chemical composition determined in our study and available literature evidence (Nasrollahi and Yadegari, 2016), the superior efficacy of silverskin extracts compared to other by-products could be attributed not only to caffeine content but also to the concurrent presence of other compounds with antimicrobial activity and potentially complementary modes of action, which could generate synergism. However, definitive proof of these compounds' individual and synergistic contributions would require bioassay-guided fractionation and testing of isolated compounds. The possible presence of melanoidins resulting from the roasting process should not be excluded either. For example, Jiménez-Zamora et al. (2015) have reported that the presence of coffee melanoidins exerts intense antimicrobial activity against pathogenic bacteria in food products, attributable to their chelating capacity. The combined presence of caffeine and melanoidins could also contribute to the observed synergistic effect.

4.2.3. Comparison with efficacies reported in the literature

The experimental results obtained for various coffee by-products

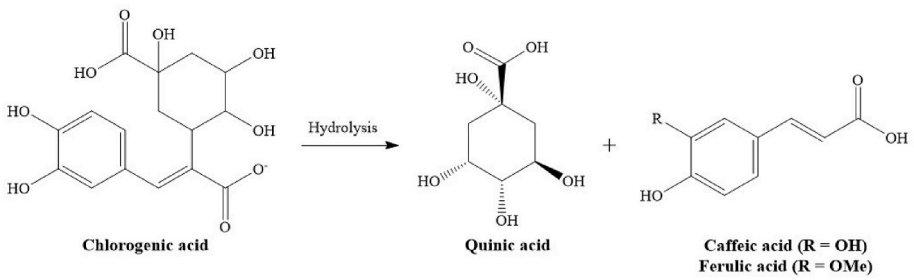

Fig. 4. Hydrolysis of chlorogenic acid.

demonstrate significant heterogeneity in their antimicrobial efficacy throughout the scientific literature (Alström, 2008; Alvarado-Ambriz et al., 2020; Bouhlal et al., 2020; Calheiros et al., 2023; Chaves-Ulate et al., 2023; Díaz-Hernández et al., 2022; Duangjai et al., 2016; Farouk et al., 2023; Mirón-Mérida et al., 2019; Monente et al., 2015; Nzekoue et al., 2020; Rodrigues et al., 2014; Sangta et al., 2021; Sousa et al., 2015; Ziemah et al., 2024), and comparisons of efficacies with other pathogens should be approached with caution as susceptibility varies depending on the pathogen, strain, extraction procedure, or by-product origin used. Table S5 presents a summary of the activities reported in the literature for the main coffee by-products against plant and human pathogens. It is noteworthy that the MICs reported in the literature for extracts of used coffee grounds, pulp, mucilage, green beans, roasted coffee, and ground coffee are typically on the order of thousands or tens of thousands of $\mu g \cdot mL^{-1}$, much higher than those reported in this work.

As for results for silverskin extracts, they are disparate: in some cases, activities comparable to those of other by-products are reported (Nzekoue et al., 2020; Ziemah et al., 2024), while in others they are much higher (Rodrigues et al., 2014). In fact, in the study by Rodrigues et al. (2014), MIC values of only 31.3 μg mL^{-1} are reported for ethanolic, hydroethanolic, and aqueous silverskin extracts against some pathogens, similar to those observed in our case.

If we focus on research employing the same pathogens as in this study, the work of Farouk et al. (2023) stands out, in which hydromethanolic extracts of spent coffee grounds were used against *Aspergillus* spp, including *A. flavus*. The MICs, in the range of 380–460 μg mL^{-1} (with the highest value corresponding to *A. flavus*) are better than those obtained in our case for husk and parchment extracts (1000 and 750 μg mL^{-1}, respectively), but worse than those of the defective green bean extract (250 μg mL^{-1}) and the silverskin extract (31.2 μg mL^{-1}).

Furthermore, in the study by Calheiros et al. (2023) investigating hydroethanolic extracts of spent coffee grounds and ground coffee against *A. niger* and *Fusarium oxysporum* Schltdl., MICs greater than 1100 μg mL^{-1} were reported for spent grounds and greater than 1770 μg mL^{-1} for ground coffee. In the same study, when working with decaffeinated ground coffee extracts and decaffeinated spent coffee grounds, the MIC values against both pathogens were greater than 1415 μg mL^{-1} for ground coffee and greater than 2400 μg mL^{-1} for spent grounds. For comparative purposes, the MICs for *A. niger* and *F. xylarioides* in this research have been in the 62.5−1000 μg mL^{-1} and 15.6–375 μg mL^{-1} range, respectively, depending on the by-product extract.

Regarding the study by Mirón-Mérida et al. (2019), in which hydroethanolic extracts of parchment were tested as antifungal additives in gellan gum films against *Fusarium* sp., the authors report that partial growth inhibition was observed at values of 8 mg cm^{-2}, but such a value is not directly comparable with MIC values, as a standard methodology was not followed.

4.3. Practical applicability and circular economy implications

The findings of this study demonstrate significant potential for implementing circular economy principles within the coffee industry. The successful extraction and application of bioactive compounds from coffee by-products addresses two critical challenges simultaneously: waste management and sustainable crop protection. With over 10 million tons of coffee waste generated annually worldwide, the valorization approach presented here transforms environmental liabilities into valuable resources.

From an economic perspective, the extraction process shows promise for commercial viability. The high caffeine content recovered, particularly from defective green beans (52 %) and silverskin (18.5 %), coupled with current caffeine market prices of 133 $€ \cdot kg^{-1}$, suggests potential revenue streams that could offset processing costs.

The demonstrated efficacy of these extracts as natural fungicides presents an environmentally sustainable alternative to synthetic pesticides. The MICs achieved (15.6–62.5 μg mL^{-1} for silverskin extract) were lower than the effective doses of commercial synthetic fungicides tested in this study, while offering the advantages of biodegradability and reduced environmental damage. This is particularly relevant in the context of increasing regulatory restrictions on synthetic pesticides and growing consumer demand for organically produced food.

The dual application potential for both crop protection and post-harvest storage represents a comprehensive approach to coffee disease management. The complete prevention of coffee tracheomycosis at 15.6 μg mL^{-1} and effective mycotoxin inhibition at 62.5−125 μg mL^{-1} demonstrate practical efficacy levels that could be implemented in commercial coffee production systems.

The comparison with commercial synthetic fungicides provides important context for the potential of these extracts. While we did not determine precise MICs for the commercial products, our silverskin extract achieved complete fungal inhibition at concentrations lower than the recommended application rates of standard commercial fungicides. Although direct comparison is limited by differences in formulation (commercial fungicides contain adjuvants and stabilizers that may enhance or reduce the amount of active ingredient needed) the inherently low effective concentrations of coffee extracts suggest promise for development. This could translate to reduced environmental loading and potentially lower application volumes, though field validation and formulation optimization remain essential next steps.

The translation of coffee by-product extracts into commercial antifungal products requires consideration of several processing factors. The aqueous ammonia extraction method employed offers advantages for industrial scale-up: it uses a relatively inexpensive and recoverable solvent, operates at ambient temperature, and yields stable freeze-dried extracts with extended shelf-life. For practical application, the extracts could be formulated as: (i) wettable powders for foliar applications, incorporating appropriate carriers and surfactants; (ii) seed treatment formulations for pre-planting protection; or (iii) post-harvest treatments for stored commodity protection. The low effective concentrations (15.6–62.5 μg mL^{-1}) suggest that formulation concentrations of 0.1–1.0 % active extract would be economically viable. Furthermore, the extraction process allows for recovery of high-value compounds like caffeine, which could offset production costs through a biorefinery approach.

4.4. Study limitations and future research directions

Several limitations should be acknowledged in this research. The GC-MS analysis failed to detect melanoidins due to their high molecular weight and low volatility, potentially underestimating the contribution of these compounds to the observed antifungal activity. Future studies should employ complementary analytical techniques such as LC-MS with electrospray ionization or derivatization protocols to better characterize these important components.

Additionally, our attribution of antifungal activity to specific compounds is based on chemical composition analysis and literature evidence rather than direct experimental validation. Future mechanistic studies should include: (i) bioassay-guided fractionation to identify and confirm active compounds; (ii) enzyme activity characterization and protein profiling in treated fungi; (iii) direct measurement of oxidative stress markers including ROS generation, lipid peroxidation, and antioxidant enzyme responses in fungi exposed to the extracts; and (iv) systematic evaluation of synergistic effects through factorial combination studies. These investigations would provide the mechanistic foundation necessary to optimize extract formulations and predict efficacy against diverse pathogen populations.

Furthermore, our comparison with commercial fungicides was limited to testing at recommended doses and one-tenth of these doses, rather than determining their precise MICs, and commercial formulations include adjuvants and delivery systems that influence field efficacy beyond the active ingredient concentration alone.

The study focused exclusively on Arabica coffee by-products from

dry processing methods. Investigation of Robusta coffee varieties and wet processing residues would provide a more comprehensive understanding of the variability in bioactive compound content and antimicrobial efficacy across different coffee types and processing methods.

The research was conducted under controlled laboratory and greenhouse conditions. Field trials are necessary to validate the efficacy of these extracts under real agricultural conditions, including the evaluation of factors such as environmental stability, application methods, natural pressure of native pathogens, and integration with existing integrated pest management systems. This validation should be coupled with the development of standardized formulations for commercial application, encompassing stability testing, shelf-life determination, and compatibility assessment with existing agricultural practices and equipment.

Economic feasibility analysis represents another critical research gap. While the high value of extracted compounds suggests commercial viability, comprehensive cost-benefit analyses are needed to determine the true economic potential of this valorization approach. This should include optimization of extraction parameters to maximize bioactive compound recovery while minimizing processing costs, evaluation of alternative extraction solvents and processing conditions, yield optimization studies, market dynamics assessment, and scale-up considerations for commercial implementation.

Finally, the broader environmental impact assessment of this valorization approach should be conducted, including a life cycle analysis comparing the environmental footprint of coffee by-product-derived fungicides versus conventional synthetic alternatives. Such analysis would provide crucial data for supporting policy decisions and industry adoption of circular economy practices in coffee production.

5. Conclusions

This study demonstrates the superior antifungal efficacy of coffee by-product aqueous ammonia extracts, with the silverskin extract exhibiting MICs of 15.6 μg mL^{-1} against *F. xylarioides*, 31.2 μg mL^{-1} against *A. flavus*, and 62.5 μg mL^{-1} against both *A. niger* and *P. verrucosum*, representing improvements of up to 24-fold compared to other coffee by-products tested. The chemical characterization revealed caffeine as the predominant bioactive component, comprising 18.5 % of silverskin extract, alongside quinic acid (1.84 %), *n*-hexadecanoic acid (6.36 %), and various phenolic compounds that may synergistically contribute to the observed antimicrobial activity.

The practical applications validated through *in planta* and *ex-situ* assays demonstrate significant potential for commercial implementation. Complete protection against coffee tracheomycosis was achieved at 15.6 μg mL^{-1}, while post-harvest protection of coffee beans prevented fungal growth of *A. flavus* and *P. verrucosum* at 62.5 μg mL^{-1}. For *A. flavus*-infected samples, this concentration also prevented mycotoxin production. Complete aflatoxin prevention in *A. niger*-infected samples, however, required a higher concentration (125 μg mL^{-1}). With effective concentrations comparable to or lower than those of tested commercial fungicides, these findings position aqueous ammonia extracts from coffee by-products as promising eco-friendly alternatives to conventional synthetic fungicides, offering dual benefits of waste valorization and sustainable crop protection while supporting the transition toward more environmentally responsible agricultural practices in the global coffee industry.

CRediT authorship contribution statement

Eva Sánchez-Hernández: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Jorge Clérigo-de Santiago:** Writing – original draft, Investigation. **Vicente González-García:** Writing – original draft, Resources, Methodology, Investigation. **Marta Herrera-Sánchez:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **José Luis Marcos-Robles:** Writing – original draft, Validation, Investigation, Conceptualization. **Jesús Martín-Gil:** Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition. **Pablo Martín-Ramos:** Writing – review & editing, Writing – original draft, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of generative AI in scientific writing

During the preparation of this work the authors used Claude.ai in order to improve the readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors report financial support was provided by Junta de Castilla y León under project VA148P23, with FEDER co-funding. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2025.107442.

Abbreviations

AFT, total aflatoxins; FTIR, Fourier-transform infrared; GC-MS, gas chromatography-mass spectrometry; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MIC, minimum inhibitory concentration; OTA, ochratoxin A; PDA, potato dextrose agar; PDB, potato dextrose broth.

Data availability

All data supporting the findings of this study are available within the paper and its Supplementary material. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

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