The sustainable cycle of bio-based plastic: from manufacturing to biodegradation procedure

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1. Manufacture of bio-based plastic and properties

Table S1. Granulometric characteristics of the cocoa bean shell powder

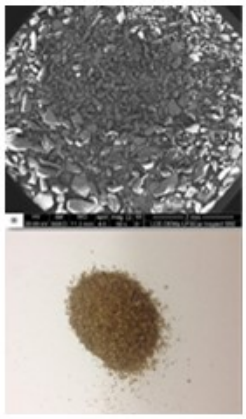
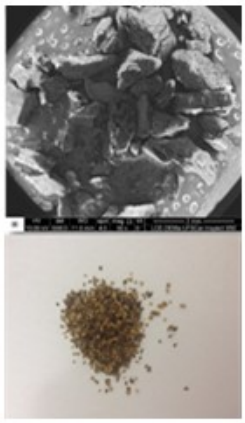
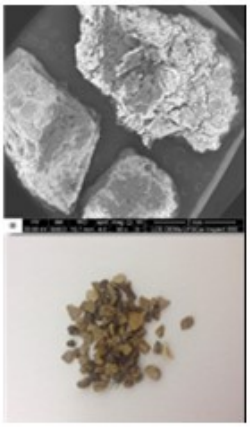
Smaller than 0.5 mm	**0.5 to 1.0 mm**	**1.0 to 2.0 mm**	**Bigger than 2.0 mm**
			
Mass percentage of each granulometric essay of the total powder obtained (%)			
13.5	24.1	46.3	16.2
Bulk Density (g/cm³)			
0.42	0.44	0.45	Not acquired
Real Density (g/cm³)			
1.442	1.445	1.515	Not acquired
Stacking Factor			
0.57	0.50	0.46	Not acquired

Table S2. Torque values for the thermoplastics and the mixtures at the end of the essay of bio-based plastics manufactured with polypropylene (PP) and polyethylene (PE) in different compositions

Composition (PP)	Torque (N.m)	Composition (PE)	Torque (N.m)
PP100	3.3	PE100	8.8
PP80/20 (1)[a]	3.7	PE80/20 (1)	10.0
PP60/40 (1)	5.8	PE60/40 (1)	14.2
PP60/40c[b] (1)	5.8	PE60/40c (1)	16.0
PP80/20 (2)[c]	3.9	PE80/20 (2)	9.8
PP60/40 (2)	6.1	PE60/40 (2)	14.6
PP60/40c (2)	5.8	PE60/40c (2)	15.2

[a] The number (1) indicates a granulometry of cocoa shells up to 0.5 mm (see Table S1).
[b] The letter c indicates the addition of a compatibilizing agent.
[c] The number (2) indicates a granulometry of cocoa shells between 0.5 to 2.0 mm (see Table S1).

Table S3. Melt Flow Index (MFI) results for extruded samples of bio-based plastics manufactured with polypropylene (PP) and polyethylene (PE) in different compositions

PP-based samples	MFI 230 ^{o}C / 2.16 kg (g/10 min)
PP100	27.7 ± 0.1
PP80/20	19.5 ± 0.3
PP80/20c[a]	16.8 ± 0.2
HDPE-based samples	**MFI 190 ^{o}C / 2.16 kg (g/10 min)**
PE100	7.2 ± 0.1
PE80/20	5.4 ± 0.1
PE80/20c	5.7 ± 0.3

[a] The letter c indicates the addition of a compatibilizing agent.

Table S4. Comparison between mechanical properties of bio-based plastics manufactured with polypropylene (PP) and polyethylene (PE) in different compositions

Sample	Young's modulus (GPa)	Tensile Strength (MPa)	Elongation (%)
PP100	0.97 ± 0.09	17.9 ± 0.4	20.2 ± 2.9
PP80/20	1.13 ± 0.37	15.5 ± 0.1	3.9 ± 0.3
PP80/20c[a]	1.25 ± 0.17	15.1 ± 0.3	3.4 ± 0.6
PE100	1.37 ± 0.05	25.5 ± 0.4	418.9 ± 4.4
PE80/20	1.14 ± 0.10	22.5 ± 0.3	7.6 ± 1.2
PE80/20c	1.9 ± 0.21	20.5 ± 0.3	8.1 ± 1.1

[a] The letter c indicates the addition of a compatibilizing agent.

2. Fungi used in biodegradation study

Colletotrichum gloeosporioides

> consensus 1649/17 - DRM 03

ATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACCTACAACTGTTGCTTCGGCGGGTAGGGTCCCCGTGACCCTCCCGGCCTCC
CGCCCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAA
AACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
CATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGG
TGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCAC
TGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGC

Figure S1. Partial sequence of the ribosomal operon of C4 (*Colletotrichum gloeosporioides*). Analyses performed at CPQBA.

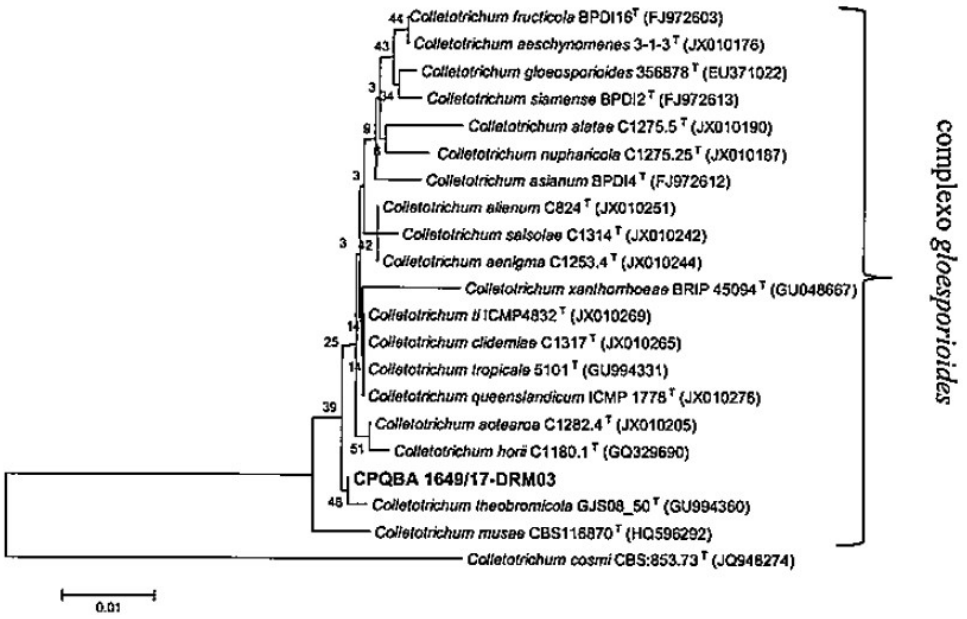


Figure S2. Phylogenetic tree demonstrating the relationships between the partial sequence of the ribosomal operon region ITS1-5.8S-ITS2 of C4 (*Colletotrichum gloeosporioides*) and sequences of strains of related microorganisms present in the databases CBS and Genbank. Analyses performed at Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA).

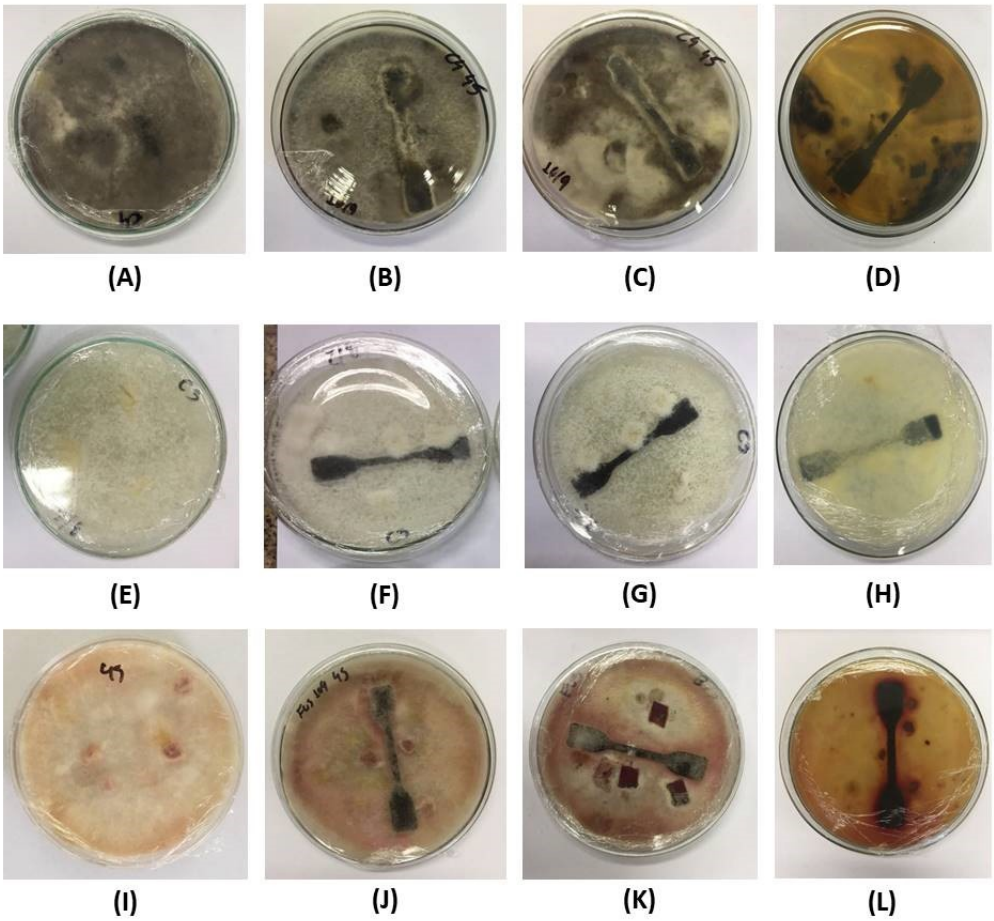


Figure S3. *C. gloeosporioides, Xylaria* sp. and *F. graminearum* cultures in different stages **a)** *C. gloeosporioides* (control) **b)** *C. gloeosporioides*-polymer interaction after 45 days **c)** *C. gloeosporioides*-polymer interaction after 75 days (frontal side) **d)** *C. gloeosporioides*-polymer interaction after 75 days (back side) **e)** *Xylaria* sp. (control) **f)** *Xylaria* sp.-polymer interaction after 45 days **g)** *Xylaria* sp.-polymer interaction after 75 days (frontal side) **h)** *Xylaria* sp.-polymer interaction after 75 days (back side) **i)** *F. graminearum* (control) **j)** *F. graminearum*-polymer interaction after 45 days **k)** *F. graminearum*-polymer interaction after 75 days (frontal side) **l)** *F. graminearum*-polymer interaction after 75 days (back side).

3. LC-MS/MS of metabolites produced by fungi

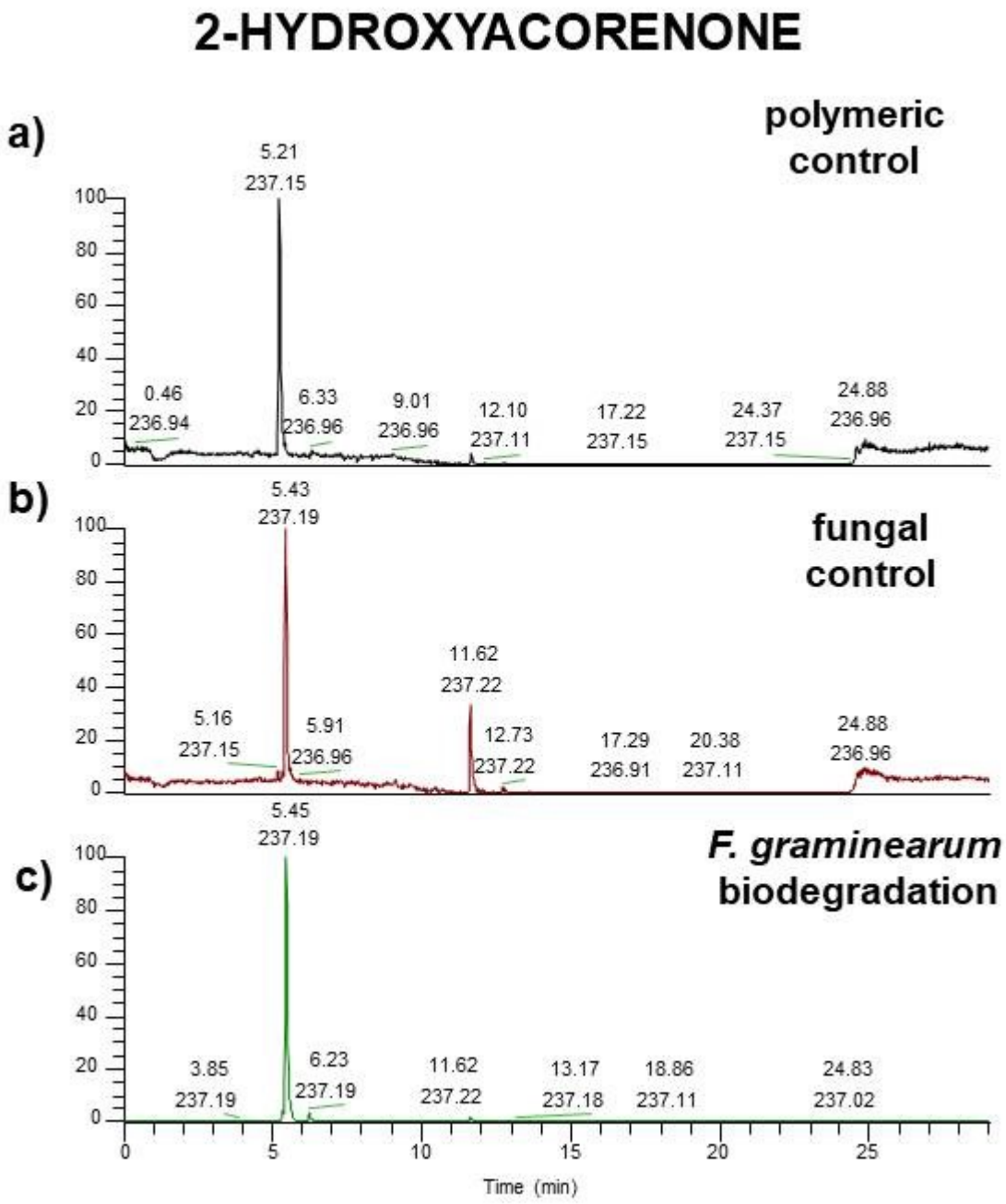
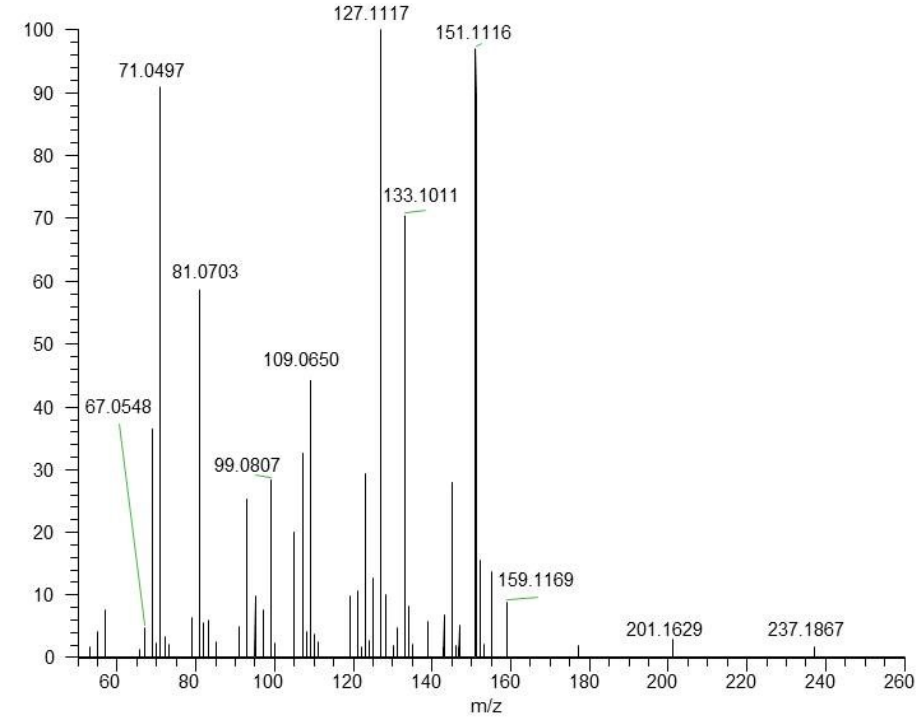
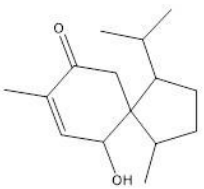


Figure S4. Ion chromatogram extracted (*m/z* 237.1843) to **a)** polymeric control; **b)** fungal control; **c)** *F. graminearum* biodegradation. LC-MS/MS spectrum of 2-hydroxyacorenone was found at retention time 5.45 min.



Database m/z (HMDB) predicted spectrum	Experimental m/z	Error (ppm)	Formula Xcalibur
53.0391	53.0392	0.42	CH_5
55.0548	55.0549	0.58	C_4H_7
57.0704	57.0341	0.39	C_3H_5O
67.0548	67.0548	0.53	C_5H_7
79.0547	79.0547	0.29	C_6H_7
81.0704	81.0703	0.44	C_6H_9
109.1017	109.1013	0.13	C_8H_{13}
121.1017	121.1012	0.01	C_9H_{13}
123.1173	123.1169	0.13	C_9H_{15}
135.1173	135.1164	0.53	$C_{10}H_{15}$
151.1122	151.1116	-0.09	$C_{10}H_{15}O$



2-Hydroxyacorenone ($C_{30}H_{18}O_{12}$)

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
237.1849	237.1849	0.14	3.5	$C_{15}H_{25}O_2$

Figure S5. LC-MS/MS spectrum of 2-hydroxyacorenone and comparison of experimental *m/z* values with predicted spectrum from HMDB (https://hmdb.ca/spectra/ms_ms/81872).

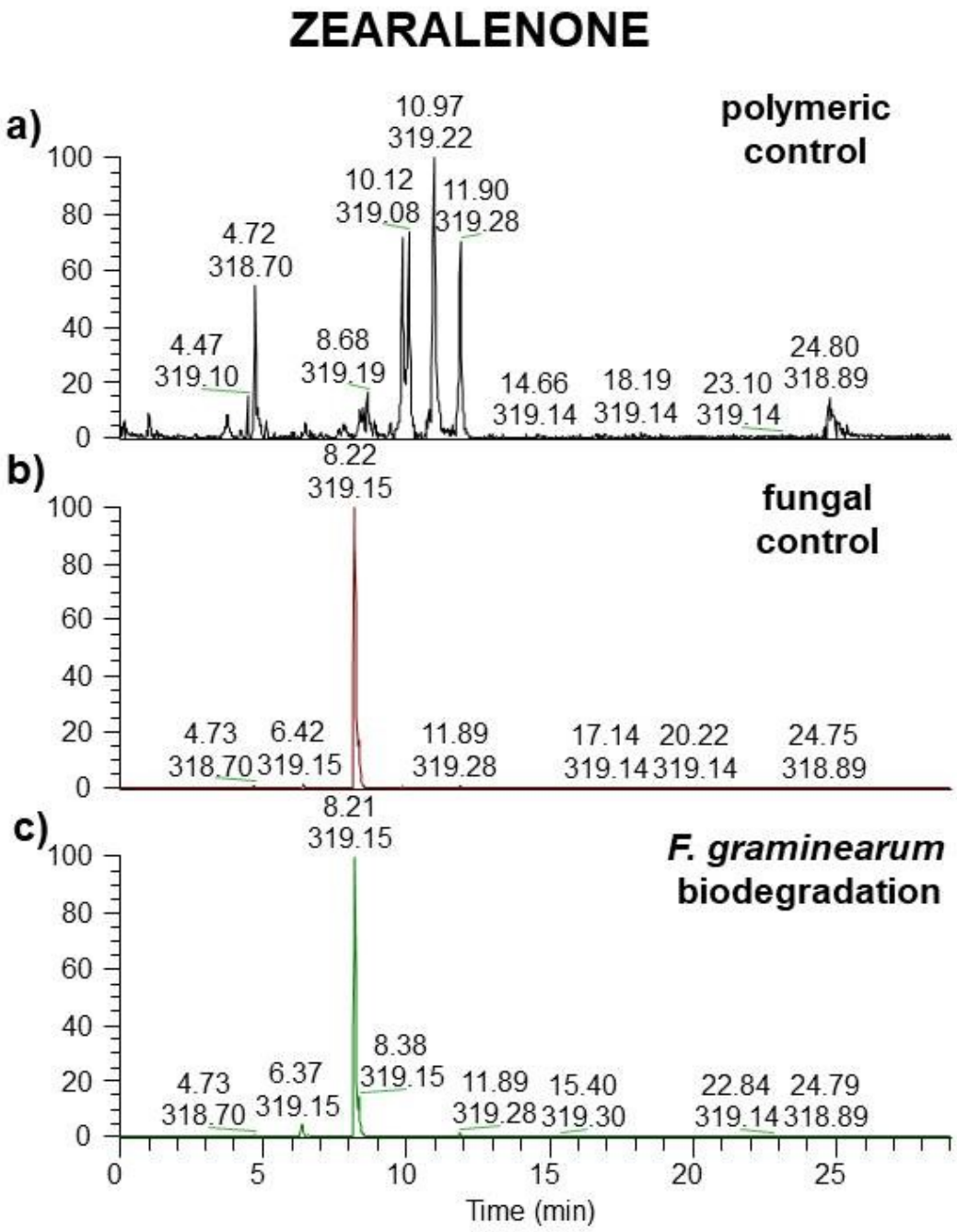


Figure S6. Ion chromatogram extracted (*m/z* 319.1540) to **a)** polymeric control; **b)** fungal control; **c)** *F. graminearum* biodegradation. LC-MS/MS spectrum of zearalenone was found at retention time 8.21 min.

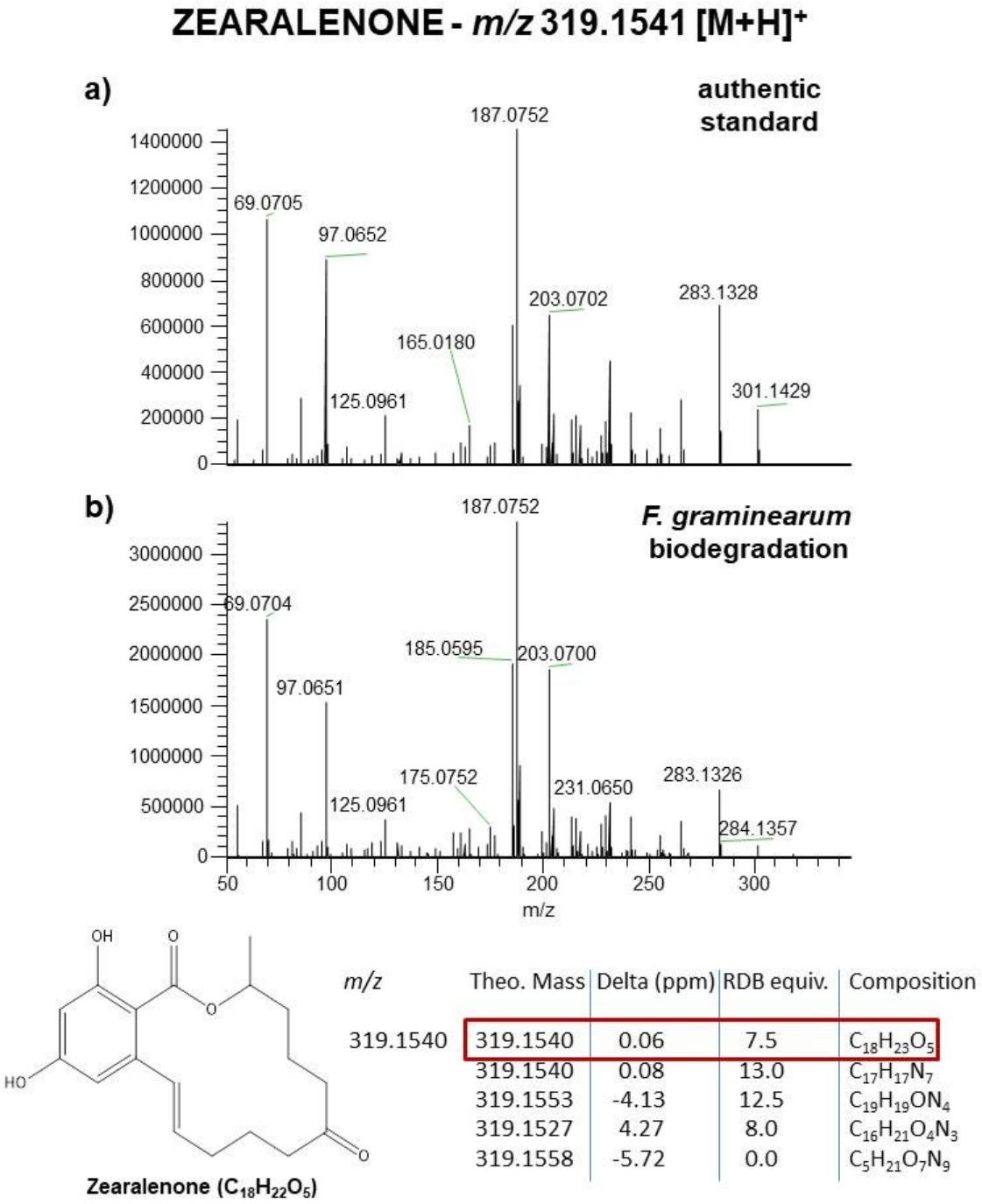


Figure S7. LC-MS/MS spectra in positive ionization mode to **a)** Authentic standard of zearalenone; **b)** *F. graminearum* biodegradation.

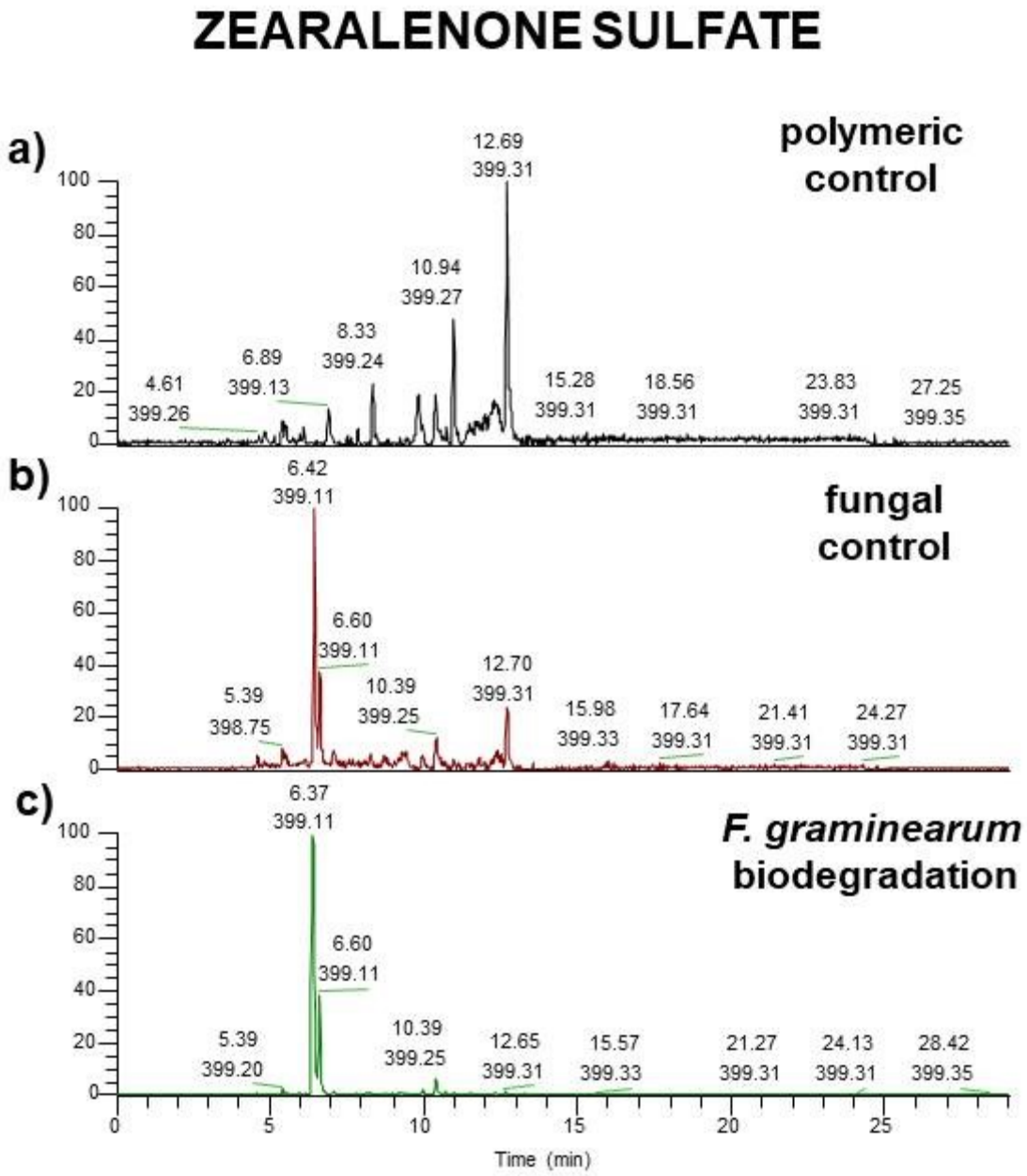


Figure S8. Ion chromatogram extracted (*m/z* 399.1108) to **a)** polymeric control; **b)** fungal control; **c)** *F. graminearum* biodegradation. LC-MS/MS spectrum of zearalenone sulfate was found at retention time 6.37 min.

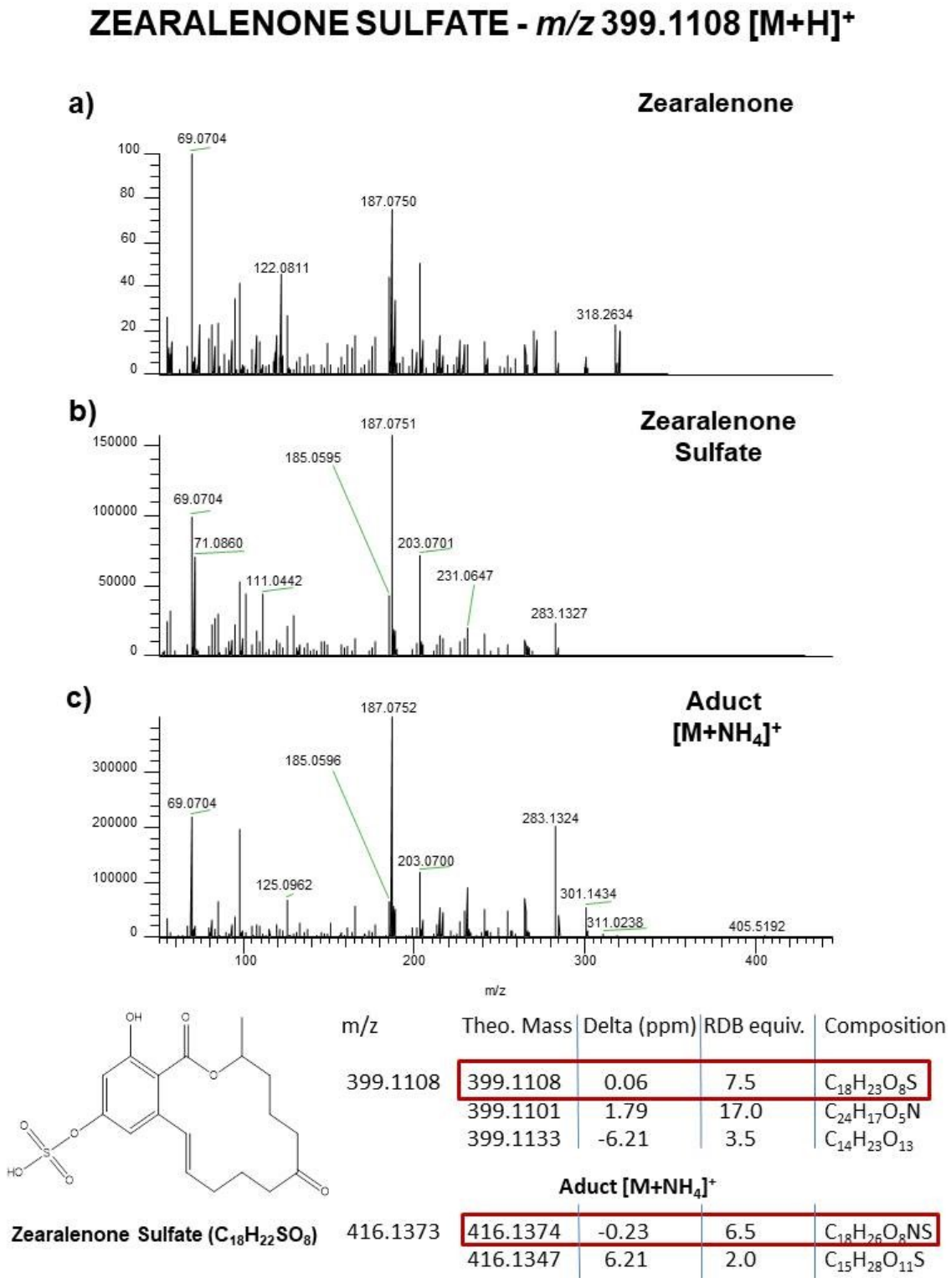


Figure S9. LC-MS/MS spectra comparison between **a)** zearalenone (*m/z* 319.1541); **b)** zearalenone sulfate (*m/z* 399.1108); **c)** the aduct $[M+NH_4]^+$ (*m/z* 416.1374).

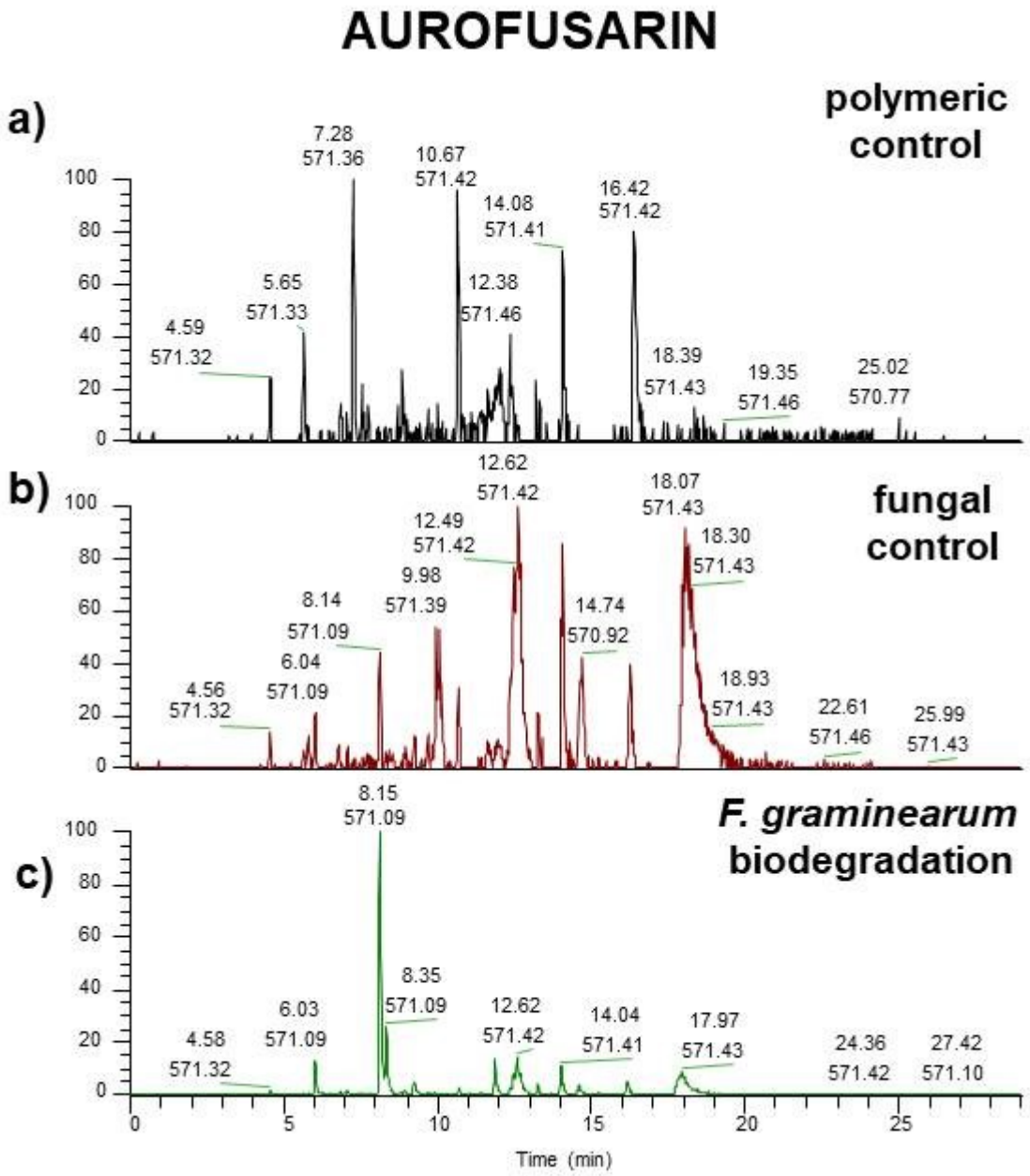


Figure S10. Ion chromatogram extracted (*m/z* 571.0869) to **a)** polymeric control; **b)** fungal control; **c)** *F. graminearum* biodegradation. LC-MS/MS spectrum of aurofusarin was found at retention time 8.15 min.

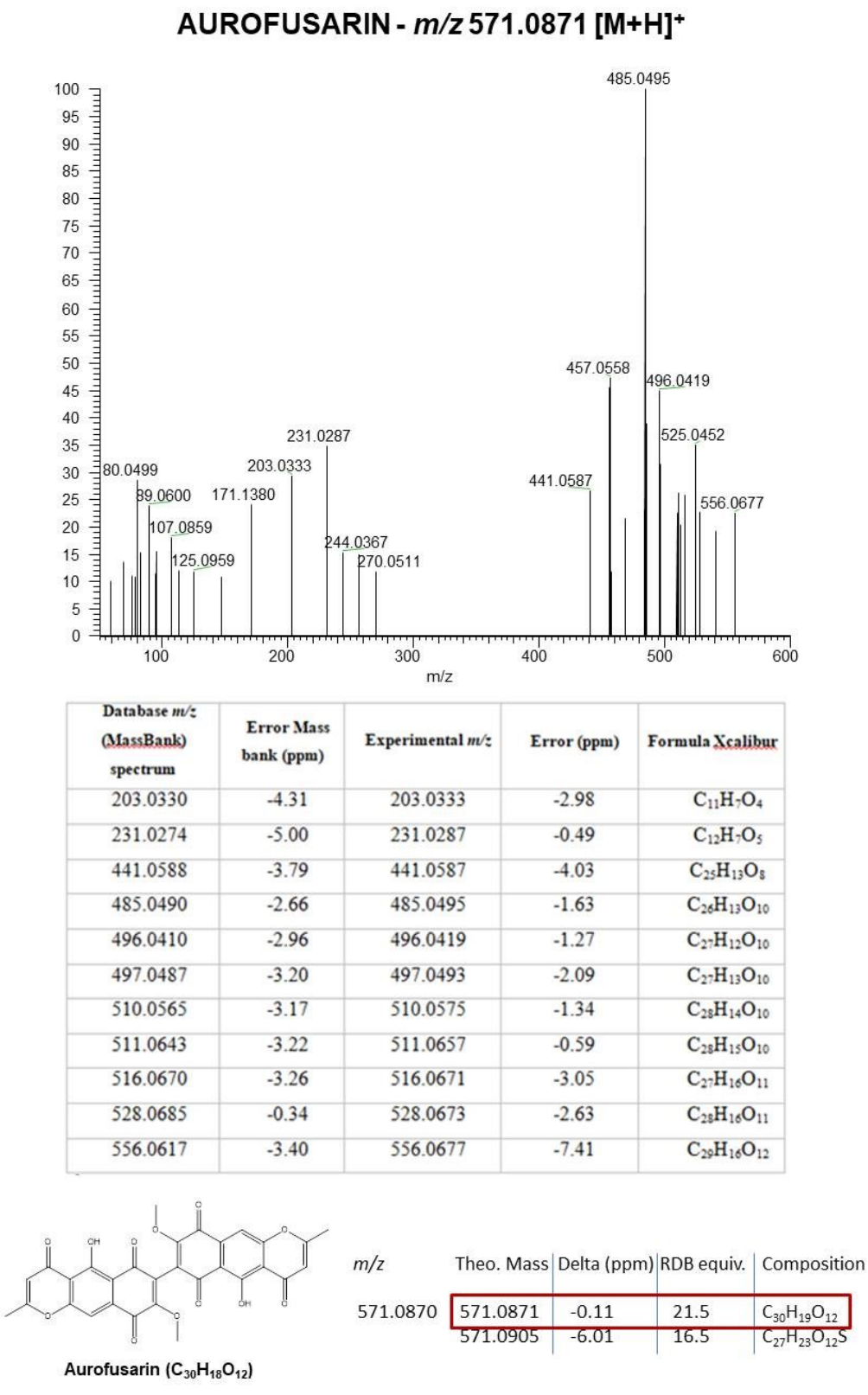
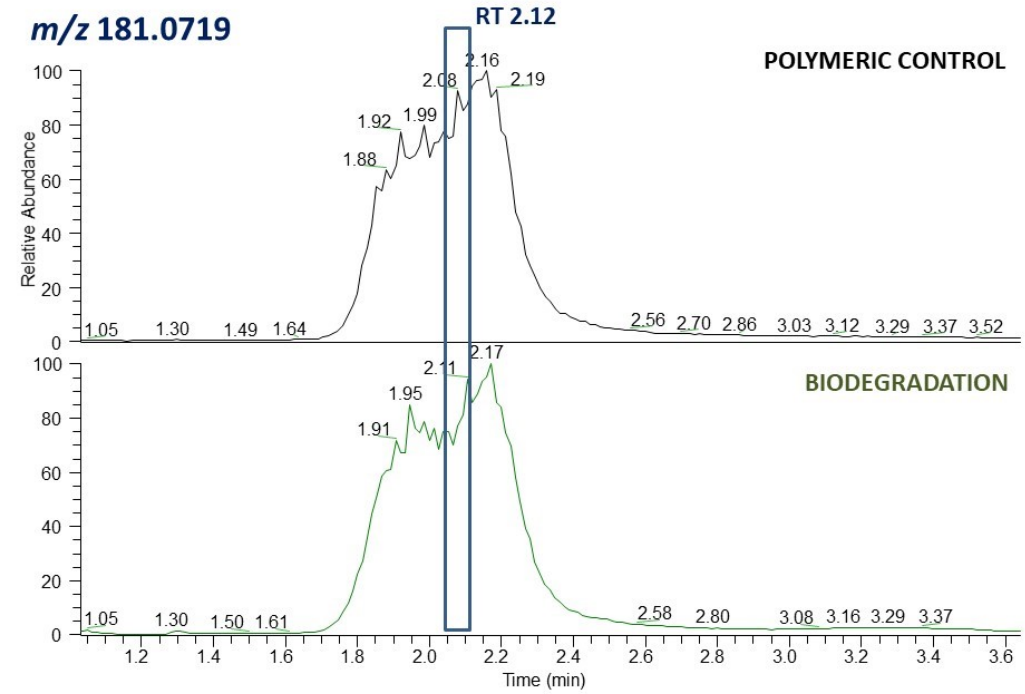


Figure S11. LC-MS/MS spectrum of aurofusarin for 35 V and comparison of experimental *m/z* values with database MassBank (https://massbank.eu/MassBank/RecordDisplay?id=AC000703).

4. LC-MS/MS of the biodegradation process



m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
181.0720	181.0720	-0.12	5.5	$C_7H_9O_2N_4$
	181.0707	7.26	0.5	$C_6H_{13}O_6$

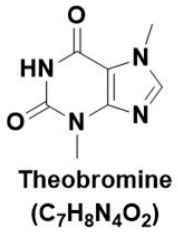
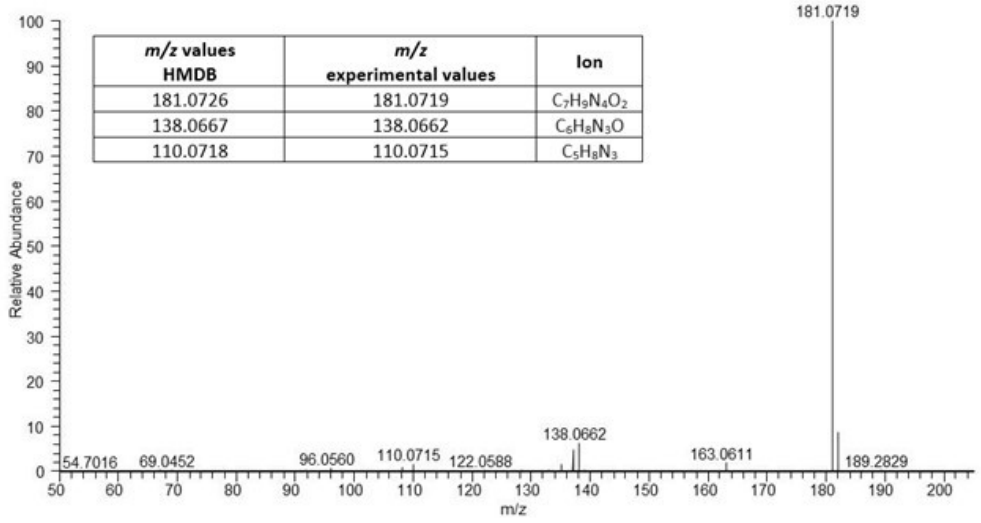
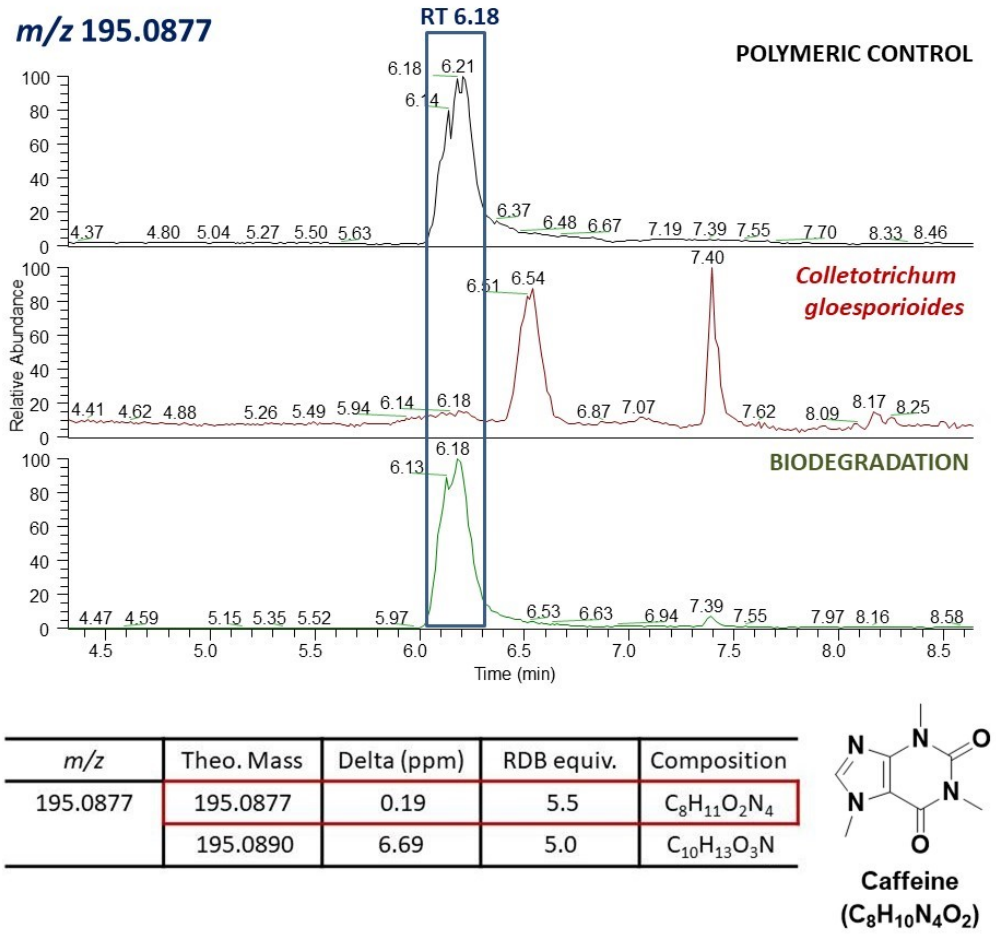


Figure S12. Chromatograms of control sample and biodegradation process using *Colletotrichum gloeosporioides*. *m/z* 181.0720 (positive mode) is related to theobromine and was found at retention time 2.12 min.



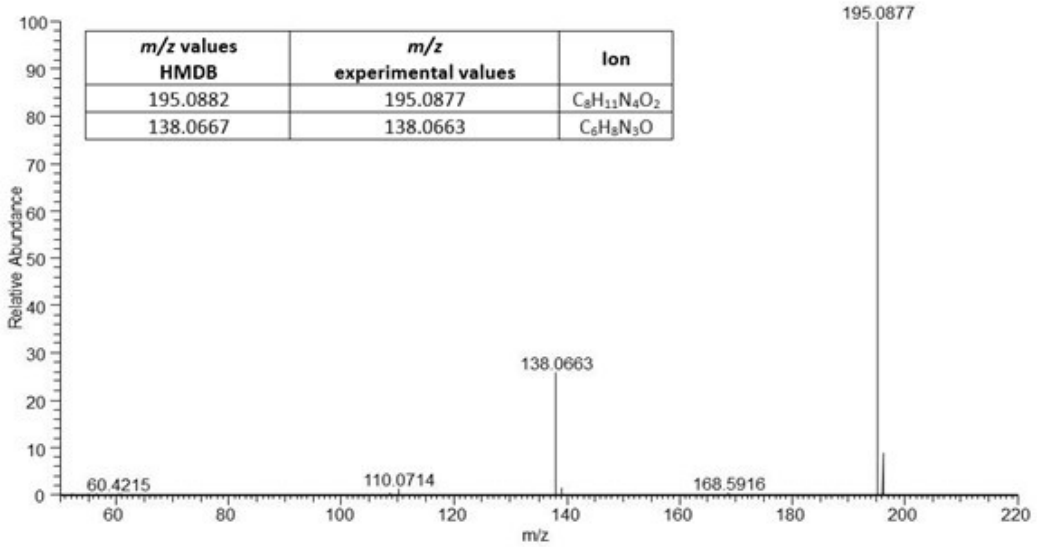
m/z values HMDB	*m/z* experimental values	Ion
181.0726	181.0719	$C_7H_9N_4O_2$
138.0667	138.0662	$C_6H_8N_3O$
110.0718	110.0715	$C_5H_8N_3$

Figure S13. LC-MS/MS (*m/z* 180.9842) in positive mode of control sample related to theobromine. Putatively annotated compound in comparison to the Human Metabolome Database (https://hmdb.ca/spectra/ms_ms/240224).



m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
195.0877	195.0877	0.19	5.5	$C_8H_{11}O_2N_4$
	195.0890	6.69	5.0	$C_{10}H_{13}O_3N$

Figure S14. Chromatograms of control sample, fungal extract of *Colletotrichum gloeosporioides* and biodegradation process. *m/z* 195.0877 (positive mode) is related to caffeine and was found at retention time 6.18 min.



m/z values HMDB	m/z experimental values	Ion
195.0882	195.0877	$C_8H_{11}N_4O_2$
138.0667	138.0663	$C_6H_8N_3O$

Figure S15. LC-MS/MS (*m/z* 195.0090) in positive mode of control sample related to caffeine. Putatively annotated compound in comparison to the Human Metabolome Database (https://hmdb.ca/spectra/ms_ms/21351).

Table S5. Relative quantification of peaks on LC-MS/MS spectra of theobromine (*m/z* 181.0720, r.t. 2.12 min) and caffeine (*m/z* 195.0877, r.t. 6.18 min) after 75 days of biodegradation

Compounds	**Measurements**	**Control**	***C. gloeosporioides***	***F. graminearum***	***Xylaria* sp.**
Theobromine	1	685,709,819	1,315,828,545	2,124,585,302	627,888,072
	2	757,268,190	1,401,761,320	2,423,704,429	426,625,349
	3	609,340,610	1,320,908,933	1,807,587,535	426,625,349
	average	684,106,206	1,346,166,266	2,118,625,755	493712923,3
	p-values		0.001	0.009	0.137
Caffeine	1	336,773,908	1,289,410,747	2,182,693,125	636,269,338
	2	432,731,451	1,451,127,228	1,817,805,552	495,072,588
	3	256,969,237	1,113,455,339	1,668,742,221	495,072,588
	average	342,158,198	1,284,664,438	1,889,746,966	542,138,171
	p-values		0.0006	0,0025	0.0266