Original Article

Uncovering Phytotoxic Compounds Produced by *Colletotrichum* spp. Involved in Legume Diseases Using an OSMAC–Metabolomics Approach

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Supplementary material

Figure S1. m/z distribution diagrams and Base Peak Chromatograms of *C. truncatum* isolate C428 (from lentil) extracts. (a) PDA extract; (b) PDB extract; (c) Rice extract; (d) Richard extract.

Figure S2. m/z distribution diagrams and Base Peak Chromatograms of *C. trucatum* isolate C431 (from soybean) extracts. (a) PDA extract; (b) PDB extract; (c) Rice extract; (d) Richard extract.

Figure S3. m/z distribution diagrams and Base Peak Chromatograms of *C. trifolii* isolate C436 (from clover extracts. (a) PDA extract; (b) PDB extract; (c) Rice extract; (d) Richard extract.

Figure S4. Structure of: validated metabolites with pure standards (level A; red); putatively identified and produced by *Colletotrichum* spp. (level B(i), blue); putatively identified and produced by other fungal species (level B(ii), black); Structures of Curvupallide A and B (Level C(i), brown).

Table S1. Parameters Metaboanalyst 5.0 for LC-MS spectra processing.

Table S2. Features lists transformation and scaling for PLS – DA analysis. Q2 and R2 values of PLS – DA models in cross validation.

Table S3. Metabolites dereplicated by targeted and untargeted metabolomics analysis, organised according to identification level.

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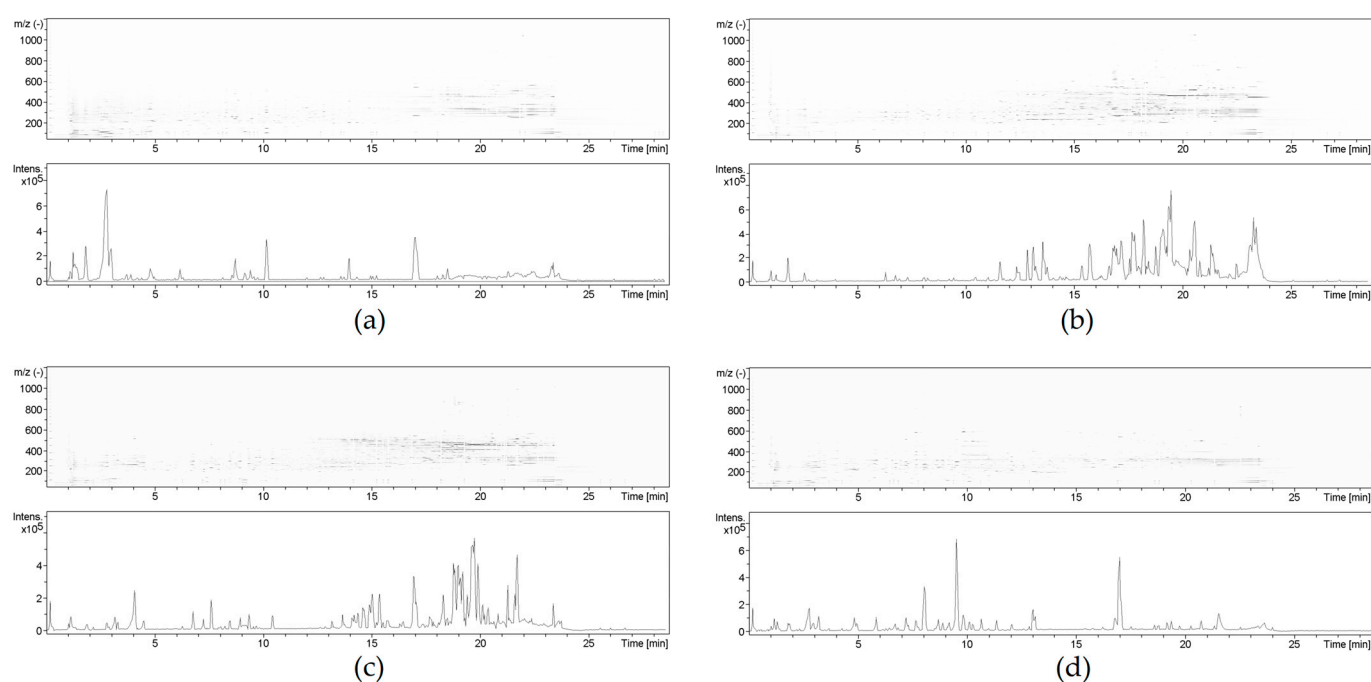
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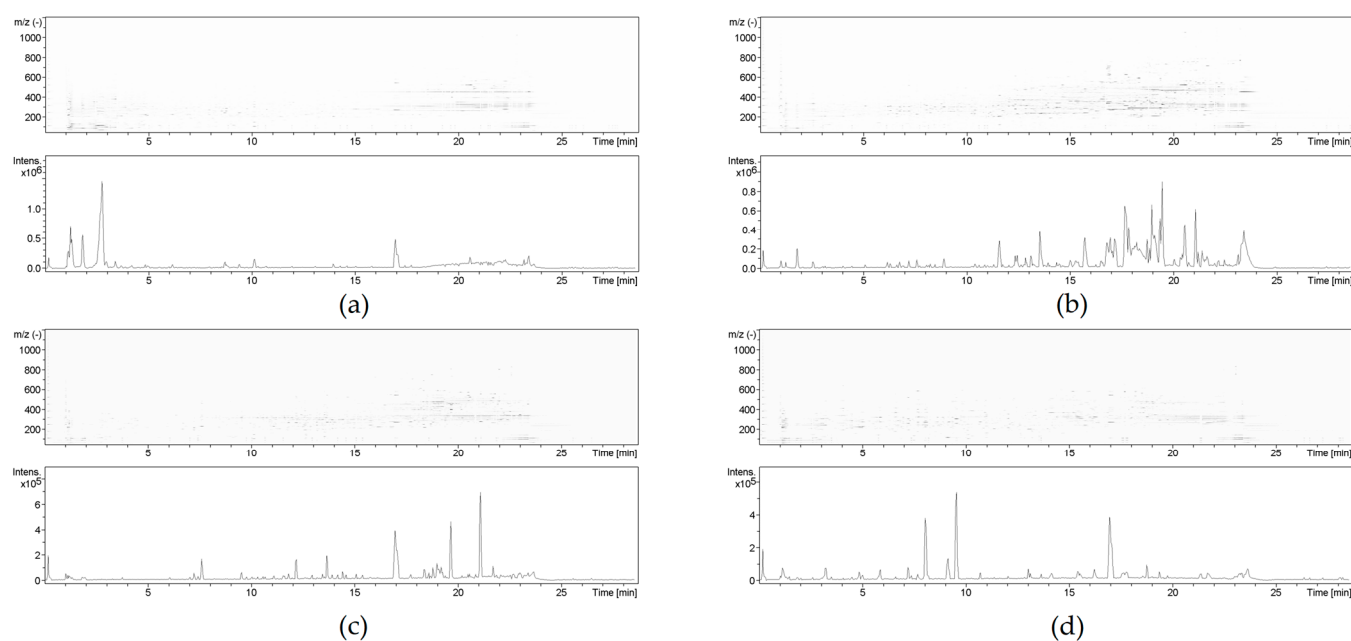

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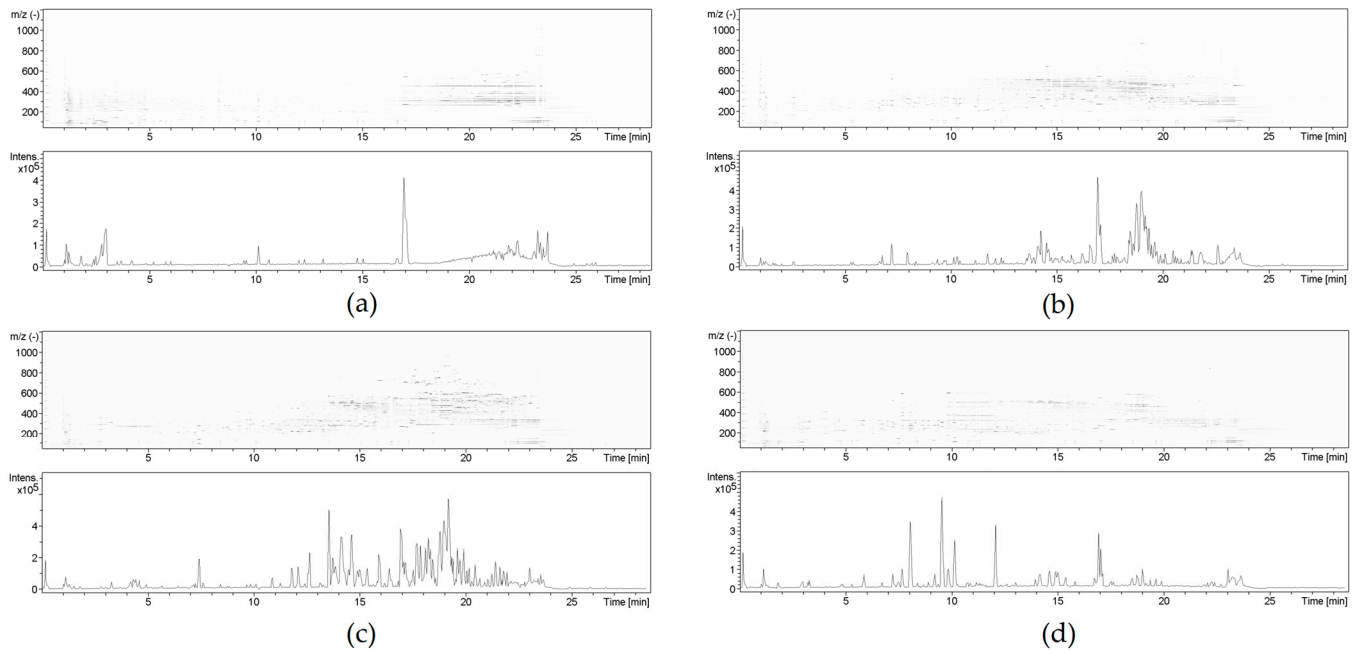
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6-Hydroxymellein

Colletopyrone

Resorcinol

4-Hydroxybenzaldehyde

Orcinol

4-Hydroxyphenyl acetic acid

Colletochlorin E

Higginsianin A

Higginsianin B

Imidazoleacetic acid

Succinic Acid

Succinic Anhydride

Maculosin

Pantothenic acid

3,4-Dehydro-6 hydroxymellein

Fusarentin 6,7-Dimethyl Ether

Colletol

Colletodiol

Macrolactone Sch-725674

Alternariol

TMC-205

3-Nitropropionic Acid

Cytosporone T

Berkchaetorubramine

Catathelasmol A

Phomolide B

Methyl 2-oxofuran-2-carboxylate

4-Chloro-3,5-dimethoxybenzaldehyde

Methyl Sydowate
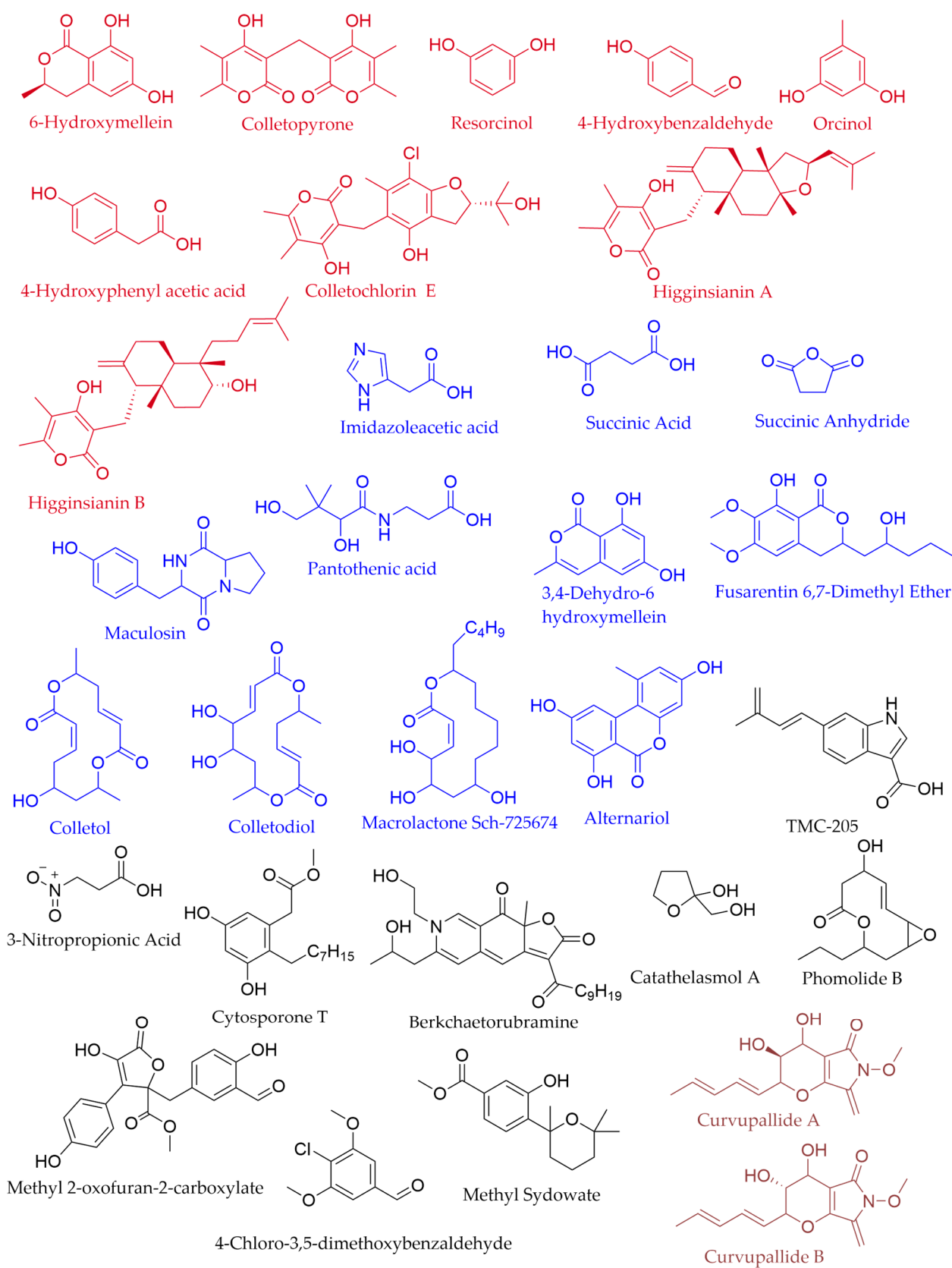
Curvupallide A

Curvupallide B

Figure S4. Structure of: validated metabolites with pure standards (level A; red); putatively identified and produced by *Colletotrichum* spp. (level B(i), blue); putatively identified and produced by other fungal species (level B(ii), black); Structures of Curvupallide A and B (level C(i), brown).

Table S1. Parameters Metaboanalyst 5.0 for LC-MS spectra processing.

Platform selection	Parameters
UPLC-Q/TOF	Auto-Optimized
Peak Picking	**Parameters**
Method	centWave
min_peakwidth	5.0
max_peakwidth	20.0
ppm	15 ppm
mzdiff	0.01
snthresh	6.0
noise	1000.0
prefilter	3.0
value_of_prefilter	100.0
Peak Alignment	**Parameters**
Method	obiwarp
Bandwidth	30.0
minFraction	0.8
minSamples	1
maxFeatures	100
integrate	1
extra	1
span	0.25
profStep	1
Peak Alignment	**Parameters**
Polarity	Negative
Adducts	$[M]^-$;$[M-H]^-$; $[M-2H]^-$; $[M-3H]^-$; $[M-H+Cl]^{2-}$; $[M+Cl]^-$; $[M+2Cl]^{2-}$;$[2M-H]^-$
Perc_fwhm	0.6
Mz_abs_iso	0.005
Max_charge	2
Corr_eic_th	0.85
Mz_abs_add	0.001
Contaminant Removal	Yes
Blank Subtraction	Yes

Table S2. Features lists transformation and scaling for PLS – DA analysis. Q2 and R2 values of PLS – DA models in cross validation.

Cultural media extracts	Transformation	Scaling	Q2 value	R2 value
PDA	Logarithmic	Pareto scaling	0.8331*	0.9900*
PDB	Logarithmic	Range scaling	0.9106**	0.9997**
Rice	Logarithmic	Pareto scaling	0.9501**	0.9998**
Richard	Cube root	Autoscaling	0.7625***	0.9967***

*values using the top 4 components; ** values using the top 5 components; ***values using the top 3 components.

Table S3. Metabolites dereplicated by targeted and untargeted metabolomics analysis, organised according to identification level.

Putative metabolite name	Biosynthetic origin and Class of compound	Molecular formula	Ret. Time	ES(-) Theor. m/z	ES(-) Found m/z	m/z error (mDa)	MS/MS	ID Levels (A – D)*	Databases Code**
6-hydroxymellein	Polyketides_ Isocoumarine	$C_{10}H_{10}O_4$	12.58	193.0501 [M-H]⁻	193.0495 [M-H]⁻	0.6	149.0601; 131.0498; 105.0715	A	CNP0395266 NPA028477
Colletopyrone	Polyketides_Cyclic polyketides	$C_{15}H_{16}O_6$	18.24	291.0869 [M-H]⁻	291.0904 [M-H]⁻	-3.5	224.0162; 139.0394; 95.0505	A	CNP0267088 Q82653372
Resorcinol	Shikimates and Phenylpropanoids_Simple phenol	$C_6H_6O_2$	10.11	109.0289 [M-H]⁻	109.0284 [M-H]⁻	0.5	93.0330; 81.0331	A	CNP0414137 Q82653372
4-Hydroxybenzaldehyde	Shikimates and Phenylpropanoids_Simple phenolic acids	$C_7H_6O_2$	9.12	121.0289 [M-H]⁻	121.0291 [M-H]⁻	-0.2	108.0200; 92.0271; 65.0407	A	CNP0230702 NPA032546
Orcinol	Shikimates and Phenylpropanoids_Simple phenol	$C_7H_8O_2$	4.76	123.0446 [M-H]⁻	123.0446 [M-H]⁻	-	109.0292; 108.0190; 91.0180; 81.0350	A	CNP0300146 NPA032537
4-Hydroxyphenylacetic acid	Shikimates and Phenylpropanoids_Simple phenolic acids	$C_8H_8O_3$	8.97	151.0395 [M-H]⁻	151.0390 [M-H]⁻	0.5	118.8785; 109.0283; 107.049; 93.0333	A	CNP0212603 NPA032547

Colletochlorin E	Terpenoids& Polyketides_Tetraketide meroterpenoids	$C_{20}H_{23}ClO_6$	18.72	393.1104 [M-H]-	393.1105 [M-H]-	-0.1	241.0638; 151.0398; 139.0400; 95.0504	A	-
Higginsianin A	Terpenoids& Polyketides_Triketide meroterpenoids	$C_{27}H_{38}O_4$	22.50	425.2692 [M-H]-	425.2686 [M-H]-	0.6	325.1839; 265.1463; 265.1478; 223.0281	A	CNP0152211 NPA021815
Higginsianin B	Terpenoids& Polyketides_Triketide meroterpenoids	$C_{27}H_{40}O_4$	22.66	427.2848 [M-H]-	427.2843 [M-H]-	0.5	357.2645; 315.2527; 283.2276; 155.0351	A	CNP0073478 NPA021814
Imidazoleacetic acid	Alkaloids_Imidazole alkaloids	$C_5H_6N_2O_2$	3.15	125.0351 [M-H]-	125.0350 [M-H]-	0.1	81.04434; 71.6317; 97.0401	B(i)	CNP0253943
Maculosin	Amino acids and Peptides_Dipeptides	$C_{14}H_{16}N_2O_3$	7.22	259.1083 [M-H]-	259.1090 [M-H]-	-0.5	98.0251; 121.0293; 124.0398	B(i)	CNP0320469 NPA007345
Succinic Acid	Fatty Acids_ Dicarboxylic acids	$C_4H_6O_4$	2.74	117.0188 [M-H]-	117.0190 [M-H]-	-0.2	99.00871; 73.0295	B(i)	Q213050
Succinic Anhydride	Fatty Acids_ Anhydride of dicarboxylic acid	$C_4H_4O_3$	2.76	99.0082 [M-H]-	99.0091 [M-H]-	-0.9	73.0138; 59.0155	B(i)	CNP0079889 Q417847
Pantothenic acid	Fatty acids_ Beta amino acids and derivatives	$C_9H_{17}NO_5$	4.94	218.1028 [M-H]-	218.1038 [M-H]-	-1	88.0413; 99.0459;	B(i)	CNP0153142

							146.0810, 71.01495		
3,4-Dehydro-6-hydroxymellein	Polyketides_ Isocoumarine	$C_{10}H_8O_4$	16.10	191.0344 [M-H]$^-$	191.0352 [M-H]$^-$	-0.8	149.0234; 147.0441; 123.0439; 87.0458	B(i)	CNP0389619 Q27105447
Fusarentin 6,7-dimethyl ether	Polyketides_ Isocoumarine	$C_{16}H_{22}O_6$	15.70	309.1338 [M-H]$^-$	309.1323 [M-H]$^-$	1.5	265.1440; 253.1075; 233.1208; 125.0243	B(i)	CNP0219353 Q27138491
Colletol	Polyketides_Macrolides	$C_{14}H_{20}O_5$	15.2	267.1235 [M-H]$^-$	267.1240 [M-H]$^-$	-0.5	223.1343; 213.1496; 121.0296	B(i)	CNP0157857 NPA002061
Colletodiol	Polyketides_Macrolides	$C_{14}H_{20}O_6$	13.7	283.1182 [M-H]$^-$	283.1187 [M-H]$^-$	-0.5	111.0822; 253.1077; 207.1020; 239.1284	B(i)	Q27896955
Macrolactone Sch-725674	Polyketides_Macrolides	$C_{18}H_{32}O_5$	18.4	327.2172 [M-H]$^-$	327.2179 [M-H]$^-$	-0.7	209.1179; 227.1282; 293.1770	B(i)	CNP0221935 NPA008159
Alternariol	Polyketides_Isocoumarine	$C_{14}H_{10}O_5$	17.53	257.0450 [M-H]$^-$	257.0457 [M-H]$^-$	-0.7	111.0087; 213.0557; 68.9974	B(i)	CNP0184929 NPA020438
TMC-205	Alkaloids_Simple indole alkaloids	$C_{14}H_{13}NO_2$	4.83	226.0868 [M-H]$^-$	226.0.884 [M-H]$^-$	-1.6	121.0294; 135.0440 167.0367	B(ii)	CNP0402436 NPA017297

3-Nitropropionic Acid	Fatty Acids_Oxo fatty acids	$C_3H_5NO_4$	2.73	118.0140 [M-H]$^-$	118.0145 [M-H]$^-$	-0.5	99.0075; 73.0281; 61.9896	B(ii)	CNP0439550 NPA007876
Cytosporone T	Polyketides_Aromatic polyketides	$C_{17}H_{26}O_4$	19.30	293.1752 [M-H]$^-$	293.1762 [M-H]$^-$	-1	275.16412; 221.15503; 236.10591; 83.05134; 149.09648	B(ii)	CNP0264849 NPA018477
Berkchaetorubramine	Polyketides_Azaphilones	$C_{27}H_{37}NO_6$	20.34	470.2543 [M-H]$^-$	470.2549 [M-H]$^-$	-0.6	414.1930; 301.1450; 251.1654; 111.0095	B(ii)	CNP0357282 NPA004011
Catathelasmol A	Polyketides_furans	$C_5H_{10}O_3$	4.58	117.0552 [M-H]$^-$	117.0553 [M-H]$^-$	-0.1	101.0246; 85.0300; 73.0304; 68.0517	B(ii)	CNP0357577 NPA010903
Phomolide B	Polyketides_Macrolides	$C_{12}H_{18}O_4$	16.08	225.1127 [M-H]$^-$	225.1134 [M-H]$^-$	-0.7	137.0970; 89.0248; 151.0764; 121.0295; 127.0764; 195.0647; 68.9970	B(ii)	CNP0206816 NPA005349

Methyl 2-[(3-formyl-4-hydroxyphenyl)methyl]-4-hydroxy-3-(4-hydroxyphenyl)-5-oxo-2,5-dihydrofuran-2-carboxylate	Shikimates and Phenylpropanoids_Lignans	$C_{20}H_{16}O_8$	16.5	351.0869 [M-H]$^-$	383.0772 [M-H]$^-$	-0.5	339.0872; 157.0136; 95.0550; 89.0252	B(ii)	CNP0354744 NPA014159
4-hloro-3,5-dimethoxybenzaldehyde	Shikimates and Phenylpropanoids_Simple phenolic acids	$C_9H_9ClO_3$	3.02	199.0162 [M-H]$^-$	199.0127 [M-H]$^-$	3.5	135.04427; 121.0297; 93.03511	B(ii)	CNP0247714 NPA002948
(+)-Methyl Sydowate	Terpenoids_Sesquiterpenoids	$C_{16}H_{22}O_4$	17.00	277.1439 [M-H]$^-$	277.1446 [M-H]$^-$	-0.7	134.0379; 127.1134; 121.0300; 111.0092; 77.0408	B(ii)	CNP0134087 NPA010996
Curvupallide A or B	Polyketides_ Isoindolazinfuranones	$C_{14}H_{17}NO_5$	10.41	278.1028 [M-H]$^-$	278.1035 [M-H]$^-$	-0.7	205.0868; 248.0924; 163.0757; 178.0858; 124.0402; 135.0445	C(i)	NPA004814/ NPA015714
3-Hydroxydodecanedioic acid; Dibutyl malate;	Fatty Acids_ Dicarboxylic acids	$C_{12}H_{22}O_5$	11.0	245.1389 [M-H]$^-$	245.1395 [M-H]$^-$	-0.6	173.0810; 111.0818; 141.0919; 103.0509	C(i)	-
Fumaric acid; Maleic acid;	Fatty Acids_ Dicarboxylic acids	$C_4H_4O_4$	2.56	115.0031 [M-H]$^-$	115.0033 [M-H]$^-$	-0.2	87.0091; 71.0139	C(i)	-

Dihydroxybutyric acid	Fatty Acids_Dihydroxy Fatty acid	$C_4H_8O_4$	1.26	119.0344 [M-H]$^-$	119.0346 [M-H]$^-$	-0.2	71.01505; 86.02505; 101.02489	C(i)	-
Hydroxybutyric acid	Fatty Acids_Hydroxy Fatty acid	$C_4H_8O_3$	2.95	103.0395 [M-H]$^-$	103.0402 [M-H]$^-$	-0.7	57.0366; 74.0233; 85.02810; 101.0232	C(i)	-
Trihydroxy octadecenoic acid	Fatty Acids_Long Chain Fatty Acids	$C_{18}H_{34}O_5$	17.54	329.2328 [M-H]$^-$	329.2335 [M-H]$^-$	0.7	229.14413; 211.1333; 171.1020; 162.02217	C(i)	-
p-Cresol; m-Cresol; o-Cresol; Benzyl alcohol; Anisole;	Shikimates and Phenylpropanoids_Simple phenol	C_7H_8O	10.1	107.0497 [M-H]$^-$	107.0504 [M-H]$^-$	-0.7	85.0304; 79.0540; 65.0380	C(i)	-
2,5-Dimethylfuran; 2-Ethylfuran	Polyketides_Furans	C_6H_8O	7.41	95.0497 [M-H]$^-$	95.0508 [M-H]$^-$	-1.1	82.0305; 68.9968; 55.0207	C(i)	-
Putative jasmonic acid derivative	Fatty acids_Octadecanoids_Jasmonic acids	$C_{12}H_{20}O_3$	19.0	211.1334 [M-H]$^-$	211.1340 [M-H]$^-$	-0.6	111.0091; 151.0762; 149. 0458	C(ii)	-
Putative colletotryptin	Alkaloids_Tryptophan alkaloids	$C_{20}H_{22}N_2O_5$	14.1	369.1450 [M-H]$^-$	369.1457 [M-H]$^-$	-0.7	325.1548; 255.0776; 221.0931	C(ii)	-
Putative glycosylamine	Carbohydrates and carbohydrate conjugate	$C_{10}H_{14}N_2O_5$	3.21	241.0824 [M-H]$^-$	241.0832 [M-H]$^-$	-0.8	223.0721; 197.0935; 144.0658; 145.0501	C(ii)	-

Putative hypoxanthine	Alkaloids _Hypoxanthines	$C_8H_{11}N_5O_3$	4.83	225.0862 [M]-	225.0885 [M]-	-2.3	165.0653; 88.0413; 82.0306; 131.0347; 113.0606	C(ii)	-
Putative colletopeptide	Cyclic peptides _ Depsipeptides	$C_{25}H_{29}N_5O_7$	14.14	510.1989 [M-H]-	510.1978 [M-H]-	1.1	494.2041; 448.1982; 281.1398; 162.0547	C(ii)	-
Putative colletopeptide	Cyclic peptides _ Depsipeptides	$C_{26}H_{29}N_3O_6$	18.31	478.1978 [M-H]-	478.2036 [M-H]-	5.0	448.1977; 281.1394; 267.1588	C(ii)	-
Putative colletopeptide	Cyclic peptides _ Depsipeptides	$C_{28}H_{42}N_4O_5$	21.70	513.3077 [M-H]-	513.3071 [M-H]-	0.6	423.2756; 358.2028; 297.2437	C(ii)	
Putative colletopeptide	Cyclic peptides _ Depsipeptides	$C_{29}H_{45}N_5O_5$	17.9	542.3342 [M-H]-	542.3335 [M-H]-	0.7	527.3226; 515.3228; 501.3428; 499.3272; 489.2980	C(ii)	-
Putative colletopeptide	Cyclic peptides _ Depsipeptides	$C_{31}H_{33}N_3O_7S$	9.82	590.1961 [M-H]-	590.1953 [M-H]-	0.8	502.1041; 444.0982; 275.1383; 178.0865	C(ii)	-
Putative benzene derivative	Benzenoids _Benzene and substituted derivatives	$C_{20}H_{18}O_3$	9.51	305.1178 [M-H]-	305.1144 [M-H]-	3.4	261.1863; 305.1752; 155.0141;	C(ii)	-

						111.0094		
Putative O-glycosil compound	Carbohydrates and carbohydrate conjugate_O-glycosil compounds	$C_{12}H_{18}O_9$	4.77	305.0873 [M-H]$^-$	305.0880 [M-H]$^-$	-0.7	306.8868; 225.0893; 181.1003; 131.0336 82.0305	C(ii)	-
Putative O-glycosil compound	Carbohydrates and carbohydrate conjugate_O-glycosil compounds	$C_{16}H_{28}O_6$	11.5	315.1808 [M-H]$^-$	315.1812 [M-H]$^-$	-0.4	283.1550; 211.0609; 68.9967	C(ii)	-
Putative phenolic glycoside	Carbohydrates and carbohydrate conjugate_Phenolic glycosides	$C_{15}H_{19}NO_8$	9.78	340.1032 [M-H]$^-$	340.1035 [M-H]$^-$	-0.3	294.0978; 221.0702; 192.0661; 138.0202; 108.0457	C(ii)	-
Putative mycosporine	Amino acids and Peptides_ Mycosporine derivatives	$C_{15}H_{17}NO_6$	6.16	306.0977 [M-H]$^-$	306.0984 [M-H]$^-$	-0.7	260.1046; 121.02924; 124.0404	C(ii)	
Putative tricarboxylic acid derivative	Carboxylic acid and derivatives_tricarboxylic acid and derivatives	$C_{12}H_{20}O_7$	17.54	275.1131 [M-H]$^-$	275.1137 [M-H]$^-$	-0.6	238.0693; 214.0169; 129.0194; 112.9852	C(ii)	-
Putative cyclic fatty acyl glycosides	Fatty Acids_ Fatty acyl glycosides	$C_{28}H_{48}O_9$	21.31	527.3220 [M-H]$^-$	527.3223 [M-H]$^-$	-0.3	470.2545; 400.2484; 348.2541;	C(ii)	-

Name	Class	Formula	RT	m/z (measured)	m/z (theoretical)	Error	Fragments		
							279.2345; 148.9299; 263.0919; 189.0532		
Putative isocoumarine	Polyketides_ Isocoumarine	$C_{11}H_{11}ClO_5$	2.76	257.0217 [M-H]$^-$	257.0252 [M-H]$^-$	-3.5	103.0389; 89.02373; 73.03042	C(ii)	-
Putative aromatic polyketide	Polyketides_Aromatic Polyketides	$C_{16}H_{16}O_5$	4.47	287.0919 [M-H]$^-$	287.0885 [M-H]$^-$	3.4	107.05028; 96.95872; 79.95595	C(ii)	-
Putative polycyclic aromatic polyketides	Polyketides_Polycyclic aromatic polyketides	$C_{18}H_{12}O_8$	16.80	355.0454 [M-H]$^-$	355.0459 [M-H]$^-$	-0.5	341.0662; 271.1446; 244.1556	C(ii)	-
Putative zearalenones	Polyketides_Zearalenones	$C_{16}H_{30}O_6$	14.20	317.1964 [M-H]$^-$	317.1967 [M-H]$^-$	-0.3	148.92999; 263.09186; 189.0532; 116.0503	C(ii)	-
Patative β amino acid derivative	Beta amino acids and derivatives	$C_9H_{12}N_2O_4$	3.67	211.0718 [M-H]$^-$	211.0727 [M-H]$^-$	-0.9	167.0816; 114.0196; 102.9327; 70.0304	C(ii)	
Putative phenolic acid	Shikimates and Phenylpropanoids_ Phenolic acids (C6-C1)	$C_{17}H_{17}NO_3$	4.76	282.1130 [M-H]$^-$	282.1096 [M-H]$^-$	3.4	192.0671; 92.05122; 148.0767	C(ii)	-

Putative phenolic acid	Shikimates and Phenylpropanoids_ Simple phenolic acids	$C_9H_9NO_4$	7.23	194.0453 [M-H]$^-$	194.0463 [M-H]$^-$	-1	150.0561; 137.0240; 108.0460; 107.0377	C(ii)	-
Putative phenylethanoid	Shikimates and Phenylpropanoids_Phenylethanoids	$C_{21}H_{32}O_{12}$	18.75	476.1893 [M-H]$^-$	476.1926 [M-H]$^-$	-3.3	460.1980; 430.1876; 237.1130; 149.0462	C(ii)	-
Putative anisol	Benzenoids _Anisoles	$C_{24}H_{31}NO_8$	19.32	460.1971 [M-H]$^-$	460.1978 [M-H]$^-$	-0.7	414.1924; 384.1817; 366.1709; 309.1538; 88.0047	C(ii)	-
Putative xanthone	Shikimates and Phenylpropanoids_Xanthones	$C_{28}H_{30}O_6$	19.60	461.1964 [M-H]$^-$	461.2009 [M-H]$^-$	-4.5	415.1001; 253.0480; 275.1405	C(ii)	-
Putative triketide meroterpenoid	Terpenoids& Polyketides_Triketide meroterpenoids	$C_{28}H_{32}O_9$	14.20	511.1968 [M-H]$^-$	511.2009 [M-H]$^-$	-4.1	421.1874; 403.1775; 375.1814; 369.1350; 299.1290	C(ii)	-
Putative terpene glycoside	Terpene glycosides	$C_{24}H_{32}NO_{10}$	14.94	494.2026 [M]$^-$	494.2030 [M]$^-$	-0.4	416.175; 369.1549; 323.1500	C(ii)	
Putative fusarin	Terpenoids_Sesquiterpenoids	$C_{23}H_{30}NO_7$	18.82	431.1944 [M-H]$^-$	431.1905 [M-H]$^-$	3.9	416.1710; 402.1710;	C(ii)	-

							389.1598; 324.1452; 303.0860	
Putative fusarin	Terpenoids_Sesquiterpenoids	$C_{23}H_{29}NO_6$	19.01	414.1917 [M-H]$^-$	414.1924 [M-H]$^-$	-0.7	387.1815; 301.1440; 352.21173; 213.12711	C(ii)	-
Putative fusarin	Terpenoids_Sesquiterpenoids	$C_{23}H_{29}NO_7$	18.56	430.1866 [M-H]$^-$	430.1873 [M-H]$^-$	0.7	400.1759; 357.1609; 320.1550; 151.0766; 139.0042; 138.0199	C(ii)	-
Putative fusarin	Terpenoids_Sesquiterpenoids	$C_{23}H_{29}NO_8$	16.41	446.1815 [M-H]$^-$	446.1821 [M-H]$^-$	-0.6	414.1925; 400.1767; 241.1453	C(ii)	-
Putative fusarin	Terpenoids_Sesquiterpenoids	$C_{23}H_{29}NO_9$	15.50	462.1764 [M-H]$^-$	462.1770 [M-H]$^-$	-0.6	448.1987; 416.1725; 323.1037; 307.1085; 196.0982	C(ii)	-
Putative fusarin	Terpenoids_Sesquiterpenoids	$C_{23}H_{31}NO_7$	18.24	432.2022 [M-H]$^-$	432.2029 [M-H]$^-$	-0.7	416.1710; 402.1710; 389.1598; 324.1452; 303.087	C(ii)	-

Putative fusarin	Terpenoids_Sesquiterpenoids	$C_{23}H_{31}NO_8$	15.12	448.1971 [M-H]$^-$	448.1977 [M-H]$^-$	-0.6	432.1654; 416.1712; 167.0717; 155.0461	C(ii)	-
Unknown	-	$C_{20}H_{32}O_9$	18.82	415.1968 [M-H]$^-$	415.1959 [M-H]$^-$	0.9	-	D	-
Unknown	-	$C_{17}H_{24}O_6$	15.50	323.1495 [M-H]$^-$	323.1502 [M-H]$^-$	-0.7	-	D	-
Unknown	-	$C_7H_{14}N_2O_4S_2$	7.42	253.0317 [M-H]$^-$	253.0341 [M-H]$^-$	-2.4	-	D	-
Unknown	-	$C_{16}H_{24}O_6$	15.03	311.1495 [M-H]$^-$	311.1499 [M-H]$^-$	-0.4	-	D	-
Unknown	-	$C_{22}H_{36}O_{13}$	16.63	508.2155 [M]$^-$	508.2187 [M]$^-$	-3.2	-	D	-
Unknown	-	$C_{26}H_{36}O_6$	16.50	524.2137 [M-H]$^-$	524.2137 [M-H]$^-$	-	-	D	-
Unknown	-	$C_{27}H_{30}O_6$	15.17	449.1964 [M-H]$^-$	449.2011 [M-H]$^-$	-4.7	-	D	-

Unknown	-		C$_{27}$H$_{35}$NO$_6$	19.4	468.2386 [M-H]$^-$	468.2386 [M-H]$^-$	-	D	-
Unknown	-		-	3.29	-	312.0872	-	D	-
Unknown	-		-	23.5	-	600.1905	-	D	-
Unknown	-		-	0.17	-	452.9227	-	D	-
Unknown	-		-	14.98	-	495.2018	-	D	-

*Confidence levels: Level A: validate metabolite. Comparison with pure standard analysed in the same experimental LC-MS/MS condition; Level B(i): Putative identification using taxonomical information. Confident match based on MS/MS data with reference standard of selected spectral libraries in MSDIAL/MSFINDER analysis (Match Score ≥ 8) and/or complete match of *in silico* fragmentation of MS/MS data of compounds restricting interrogation to *Colletrotrichum* species using MetFrag; Level B(ii): Putative identification. Complete match of *in silico* fragmentation of MS/MS, using MetFrag data restricting interrogation to fungal metabolites. Level C(i): Tentative structure. MS/MS data in agreement with different structural isomers of reference standards of selected database in MSDIAL/MSFINDER belonging to the same natural compound class; Level C(ii): Compound Class. Partial match of MS/MS data on the spectral libraries used in MSDIAL/MSFIDER (6 ≤ Match Score < 8) with specific metabolites class and/or MS/MS data in agreement with a specific class of fungal metabolites according to *in silico* fragmentation using MetFrag; Level D: Only molecular formula and/or High-resolution mass.

**Databases identifiers: ID starting with CNPXXX are Coconunt ID: https://coconut.naturalproducts.net/; ID starting with NPAXXX are Natural Product Atlas ID: https://www.npatlas.org/; ID starting with QXXX are Lotus ID: https://lotus.naturalproducts.net/.

Table S4. Metabolites dereplicated by targeted and untargeted metabolomics analysis, organised according to identification level.

Fungal species	Legume species tested	N[a]	DA[a]	NR[a]	%DS 1 mg/ml	%DS 2mg/ml	N	DA	NR	%DS 1 mg/ml	%DS 2 mg/ml	N	DA	NR	%DS 1 mg/ml	%DS 2 mg/ml	N	DA	NR	%DS 1 mg/ml	%DS 2 mg/ml
				Richard					PDA					PDB					Rice		
C. truncatum isolate C428	Lentil	+	++	+	100	100	+	++	+	89.7±14.5	100	+	++	+	72.8±19.5	84.7±14.2	+	+	-	23.7±9.6	51.8±16*
	Soybean	-	+	+	11.9±5	12.2±4.8	+	-	-	4.4±1.6	4.6±1.9	+	-	-	3.4±0.9	3.6±1.1	+	-	-	7.1±1.5	8.5±1.3
	Faba bean	+	-	-	4.3±2.5	9±2.1*	+	-	-	4.3±2.1	2.9±1	+	+	-	2.4±1.6	6.4±2.4*	+	-	-	11.6±2.3	19±4.8*
	Pea	-	+	+	20.2±8.3	26.5±4.8	+	+	-	4.4±2.3	5.9±2.7	-	+	-	4.2±2.3	35±14*	+	+	+	9.3±4.2	8.4±5.9
	Red clover	-	+	+	35.5±10.5	80.6±16*	-	+	-	31±8.9	34.4±11.4	-	+	+	27.1±6.5	28.7±2.8	-	+	+	42±13.4	72.1±18.5*
	White clover	-	+	+	35±5.7	43.5±7.3*	-	+	+	58.7±22	51.4±18	-	+	+	36.6±7.4	35.7±10.4	-	+	+	41.3±6.1	39.9±3.4
	Subterranean clover	-	+	+	86.4±12.6	89.5±9	-	+	+	16.7±2.1	44.8±8.5*	-	+	+	30.9±3.7	32.4±1.8	+	+	+	23.9±4.9	49.7±11.4*
	Barrel medic	-	+	+	94.3±6.4	100	-	+	-	89±12.5	93.5±8.4	-	+	+	92.8±12	100	-	+	+	100	96.5±4.1
C. truncatum isolate C431	Lentil	+	++	+	66±24	84.4±8.8	+	++	-	100	97.6±2.4	-	+	-	49.9±11.9	47±21.7	-	+	+	4.5±2.6	7.8±4
	Soybean	+	+	+	6.40±1.3	9.48±2.8*	+	+	+	9.8±4.7	10.5±3	+	+	+	8.4±3.6	8±3.2	+	+	+	5±2.1	7.2±1.8
	Faba bean	+	-	-	5.6±2.3	5.9±1.5	+	-	-	3.7±0.9	4.2±0.1	+	-	-	3.5±2	3.4±0.2	+	-	-	7.1±1.3	13.3±5.5*
	Pea	+	-	-	2.1±1.1	1.9±0.6	-	+	-	0.98±0.4	0.9±0.4	-	+	-	6.2±1.1	5.4±1.6	-	+	-	1.2±0.9	3±1.1*
	Red clover	+	+	+	33.4±12.5	51.8±17*	-	+	-	56.6±17	61.9±14.7	-	+	+	41.5±14	85±15*	+	+	+	30.6±10.1	38.7±12.8
	White clover	+	+	+	27.6±3.9	33.2±5.8	-	+	-	43.9±7.5	91±8.3*	-	+	-	25±13	47±14.8*	-	+	+	18.1±8.6	29±7.8*
	Subterranean clover	-	+	+	51.3±8	54.1±18.2	-	+	-	90.8±10	98.1±3.3	-	+	+	57.4±16.3	92.4±11*	-	+	+	42.6±11.7	86.9±12.2*
	Barrel medic	-	+	+	93.1±8.1	92.7±5.4	-	+	-	45.9±14.2	44.1±2.3	-	+	-	47.8±11.9	43.6±0.9	+	+	+	92.1±7	90.2±5.7
C. trifolii C436	Lentil	-	++	+	35.4±14.4	95.6±4*	-	+	-	0.1±0.1	4.4±1.6	+	+	-	11.5±3.2	26.2±4.3*	-	+	-	4.7±0.9	15.2±2.8*
	Soybean	+	+	+	6.4±1.1	9.5±2.8*	+	+	+	5.4±1.8	5.8±2.3	-	+	+	7.2±3.3	6±2.5	+	-	-	13.2±3	10.2±0.7
	Faba bean	+	+	+	11.5±2.7	17.2±5.8	+	-	-	13.2±6.6	10.6±1.2	-	+	-	6.4±2.8	5.9±1.8	+	+	-	7.8±3.6	46.7±4.7*
	Pea	+	+	+	21±10.5	57±14.9*	+	-	-	2.4±0.9	2±0.4	+	-	-	1±0.5	2.5±0.9	+	-	-	3.2±1.4	9.6±3.9*
	Red clover	+	+	+	74.2±17.6	83.7±8.7	-	+	+	52.1±19	46.1±17	+	-	-	24.7±9.7	21.7±4.5	+	+	+	42.9±19.6	51.7±17

	White clover	+	+	+	53.3±5.6	85.3±16*	-	+	+	16.7±9.3	14±3.9	-	+	+	9±1.7	10.3±3	+	+	+	17.7±2.4	90±5.7*
	Subterranean clover	+	+	+	90.4±8.9	88±9.2	+	+	+	20±3.9	14±5.6	+	+	+	37.5±16	66±13*	+	+	+	51.8±12	90.6±9*
	Barrel medic	+	+	+	100	100	-	+	+	82.7±14.3	84.4±13.9	-	+	+	39.7±11.7	77.5±19*	+	+	+	45.7±8.1	42.1±2.6
CT	Untrated	-	-	-	0	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0	0
	Water	-	-	-	0	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0	0
	MeOH 5%	-	-	-	0	0	-	-	-	0	0	-	-	-	0	0	-	-	-	0	0

a:(N) necrotic leaf area, (DA) irregular discolored areas surrounded (or not) by a (NR) necrotic ring;

* %DS at 1 mg/ml and at 2 mg/ml is significantly different ($p < 0.05$)