

### Antimicrobial volatiles of the insect-pathogen *Metarhizium brunneum*

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**Table S1.** *Metarhizium brunneum* volatile organic compounds (VOCs) tested for biocidal activity.

VOC (purity)	Synonyms	CAS Number	MW (g/mol)	Molecular formula
Isoamyl alcohol (99%)	3-Methyl-1-butanol, Isopentyl alcohol	123-51-3	88.15	C <sub>5</sub> H <sub>12</sub> O
Isoamyl formate (95%)	Isopentyl formate, 3-Methylbutyl formate, Isoamyl methanoate, Isopentyl methanoate, formic acid isoamyl ester	110-45-2	116.16	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>
Methyl isovalerate (98%)	Methyl Isopentanoate, Methyl 3-methylbutanoate, Isovaleric acid methyl ester	556-24-1	116.16	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>
3-Octanone (98%)	Ethyl amyl ketone, Ethyl pentyl ketone	106-68-3	128.21	C <sub>8</sub> H <sub>16</sub> O
(R)-(+)-Limonene (97%)	(+)-p-Mentha-1,8-diene, (+)-Carvene, (R)-4-Isopropenyl-1-methyl-1-cyclohexene	5989-27-5	136.23	C <sub>10</sub> H <sub>16</sub>
Isovaleric acid (99%)	3-Methylbutanoic acid, 3-Methylbutyric acid	503-74-2	102.13	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>
1-Octen-3-ol (98%)	Pentyl vinyl carbinol	3391-86-4	128.21	C <sub>8</sub> H <sub>16</sub> O
Farnesene (mixture of isomers)	$\alpha$ -Farnesene, 3,7,11-trimethyl-1,3,6,10-dodecatetraene	502-61-4	204.35	C <sub>15</sub> H <sub>24</sub>
(-)- $\alpha$ -Cedrene (95%)	(1S,2R,5S)-2,6,6,8-Tetramethyltricyclo[5.3.1.0 <sup>1,5</sup> ]undec-8-ene	469-61-4	204.35	C <sub>15</sub> H <sub>24</sub>
2,3-Butanediol (98%)	2,3-Butylene glycol	513-85-9	90.12	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>
1-Octene (98%)	Octene-1, Octylene; n-octene, Hexylethylene, Oct-1-ene	111-66-0	112.21	C <sub>8</sub> H <sub>16</sub>
Undecane (99%)	n-Undecane, Hendecane	1120-21-4	156.31	C <sub>11</sub> H <sub>24</sub>
Tridecane (99%)	n-Tridecane	629-50-5	184.36	C <sub>13</sub> H <sub>28</sub>
Damascenone (98%)	$\beta$ -Damascenone, 1-(2,6,6-Trimethylcyclohexa-1,3-dien-1-yl)-2-buten-1-one	23696-85-7	190.28	C <sub>13</sub> H <sub>18</sub> O

## Supplementary information

**Table S2.** Bacterial, fungal and oomycete targets used to test VOC potency.

Microbe	Group	Ecological significance	Source
<i>Micrococcus luteus</i> , NCIB 8553	Gram +	Widespread (soil, water, skin flora)	Llinos Harris, Swansea University
<i>Escherichia coli</i> , NCIB 8277	Gram –	Human Intestine	Llinos Harris, Swansea University
<i>Pantoea agglomerans</i> , NCTC 9381	Gram –	Widespread (soil, plants, insect gut), plant pathogen (fire blight)	Llinos Harris, Swansea University
<i>Bacillus megaterium</i> , NCIB 10342	Gram +	Soil bacterium, BCA use to control plant diseases and plant parasitic nematodes.	Llinos Harris, Swansea University
<i>Bacillus subtilis</i> , NCIB 3610	Gram +	Soil bacterium, commensal in human gut	Llinos Harris, Swansea University
<i>B. thuringiensis</i> subsp. <i>Galleria</i> (H-serotype V) strain 69-6	Gram +	Soil bacterium, BCA used to control insect pests	ISEA bacterial collection
<i>Pseudomonas aeruginosa</i> , PA01	Gram –	Widespread (soil, water, skin flora)	Angus Buckling, Oxford University
<i>Staphylococcus aureus</i> , ATCC 6538P	Gram +	Human and animal surfaces	American Type Culture Collection
<i>Candida albicans</i> , NCYC 3778	Yeast	Human pathogen	National Collection of Yeast Cultures
<i>Candida glabrata</i> , NCYC 3537	Yeast	Human pathogen	National Collection of Yeast Cultures
<i>Pythium ultimum</i>	Oomycete	Plant pathogen (Damping off)	Jane Nicklin, University of London
<i>Botrytis cinerea</i>	Fungus	Plant pathogen (Gray mould)	Naresh Magan, Cranfield University
<i>Fusarium graminearum</i>	Fungus	Plant pathogen (Cereal head blight)	Naresh Magan, Cranfield University

**Table S3.** *Metarhizium brunneum* VOCs identified by NIST . The list shows compounds produced independent of strain, developmental stage (mycelium vs conidia), production *in vivo* or *in vitro*. The list includes compounds produced consistently as well as those produced intermittantly.

Compound	MW (g/mol)	Compound	MW (g/mol)
<b>Alkanes</b>		<b>Esters</b>	
Pentane	72.15	Ethyl acetate	88.10
Heptane	100.21	Isoamyl formate	116.15
Octane	114.23	Methyl isovalerate	116.15
1-Methyl-2-pentylcyclohexane	168.32	Isoamyl acetate	130.18
Dodecane	170.34	Methyl (3-hydroxycyclopentyl) acetate	158
3-Methyltridecane	198.39	Methyl 2-ethylhexanoate	172.26
5-Methyltridecane	198.38	<b>Monoterpenes</b>	
Tetradecane	198.39	Limonene	136.24
7-Methylpentadecane	226.44	Limonene-6-ol pivalate	236.35
Hexadecane	226.44	<b>Sesquiterpenes</b>	
Eicosane	282.55	Acoradiene	204.35
3-Methyleicosane	296.57	Bergamotene	204.35
<b>Alkenes</b>		Bisabolene	204.35
1-Octene	112.24	Cardinene	204.35
1,3-Octadiene	110.2	Cedrene	204.35
2-ethenyl-1,3,3- trimethylcyclohexene	150.26	Chamigene	204.35
1-Undecene	154.29	Copaene	204.35
4,4-dimethyl-2-neopentyl-1-pentene	168.31	Cubebene	204.35
<b>Alcohols</b>		Di-epi- $\alpha$ -cedrene	204.35
Isoamyl alcohol	88.14	Elemene	204.35
2,3-butanediol	90.12	Farnesene	204.36
2-Phenylethanol	122.16	Himachala-2,4-diene	204.35
1-Octen-3-ol	128.22	Himachalene	204.35
<b>Ketones</b>		Isocaryophyllene	204.35
3-Octanone	128.21	Isoledene	204.35
5,9-dimethyl-5,8-decadiene-2-one	180	Patchoulene	204.35
<b>Carboxylic acids</b>		$\beta$ -sesquiphellandrene	204.35
Acetic acid	605	Thujopsene	204.35
Isovaleric acid	102.13	Valencene	204.35
2-Methylhexanoic acid	130.18	$\alpha$ -seinenene	204.35
<b>Other compounds</b>		$\alpha$ -zingiberene	204.35
3-Hydroxy-2-butanone	88.10	Cubenol	222.37
Methoxybenzene	108.14	$\alpha$ -bisaboial	222.37
Dimethoxybenzene	138.17	Damascenone	190.29

# Supplementary information

**Table S4.** VOCs produced by *Metarhizium brunneum* ARSEF4556, ARSEF3297 and V275 on different substrates. The percentage values are proportional to the compound with the highest peak (100%) in the chromatogram. (Values are means of 5 replicates). Abbreviations: Rt = Retention time, MW = Molecular weight, OSM = Osmotic Stress Medium, HCN = High C:N medium, ICN = Intermediate C:N medium, LCN = Low C:N medium, – = Not observed in this medium, \* = Authentic compounds having the same RT and MS as synthetic standard, Tr. = Trace amount.

Compound	R <sub>t</sub> (min)	MW	ARSEF 4556 (After 7 days – no conidia)				ARSEF 4556 (After 14 days – conidia)				V275 (After 7 days)				V275 (After 14 days)				ARSEF 3297 (After 7 days)				ARSEF 3297 (After 14 days)			
			OSM	HCN	ICN	LCN	OSM	HCN	ICN	LCN	OSM	HCN	ICN	LCN	OSM	HCN	ICN	LCN	OSM	HCN	ICN	LCN	OSM	HCN	ICN	LCN
Pentane*		72	-	-	-	-	65.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate*		88	-	-	-	-	-	-	-	-	-	43.04	37.95	16.42	-	-	-	-	-	-	-	-	-	-	-	-
Acetic acid*		60	-	100	100	100	-	-	-	-	100	85.76	-	-	-	-	-	-	100	100	100	100	-	-	-	-
Heptane*	1.534	100	-	-	-	-	22.60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-hydroxy-2-butanone* (Acetoin)	2.015	88	-	-	-	-	-	-	-	-	-	49.38	33.58	32.25	-	57.9	73.73	100	-	-	-	-	-	-	-	-
Isoamyl alcohol*	2.075	88	100	-	-	-	61.85	-	-	-	2.30	20.44	100	100	-	-	-	-	34.29	-	-	-	-	-	-	-
Isoamyl formate*	2.770	116	-	-	-	-	-	-	-	-	-	-	4.88	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl isovalerate*	2.945	116	-	-	-	-	-	-	-	-	-	-	2.01	-	-	-	-	-	-	-	-	6.73	-	-	-	-
1-Octene*	3.520	112	-	-	-	-	-	0.86	1.62	-	-	-	-	-	11.13	2.67	102	3.89	-	-	-	-	8.27	-	6.62	-
Octane*	4.123	114	-	-	-	-	10.70	0.57	1.12	25.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2,3-butanediol* (R or S isomer)	4.168	90	-	-	-	-	-	-	-	-	-	Tr.	Tr.	-	-	43.43	44.49	4.16	-	-	-	-	-	-	-	-
2,3-butanediol* (R or S isomer)	4.479	90	-	-	-	-	-	-	-	-	-	100	65.96	-	-	100	100	19.48	-	-	-	-	-	-	-	-
1,3-Octadiene	4.662	110	-	-	-	-	-	18.39	36.40	100	-	-	-	-	56.20	14.92	88.05	34.14	-	-	-	-	100	-	100	-
Isovaleric acid*	4.667	102	7.79	9.84	11.82	8.78	-	-	-	-	8.46	20.40	-	-	-	-	-	-	8.91	4.79	4.18	12.97	-	-	-	-
Isopentyl acetate*	4.708	130	-	-	-	-	-	-	-	-	-	105	9.40	7.5	-	-	-	-	-	-	-	-	-	-	-	-
2-Methylhexanoic acid	5.462	116	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.04	2.31	-	3.76	-	-	-	-
1-Octen-3-ol* (R, S isomer)	7.099	128	-	-	-	-	-	-	-	-	-	-	-	-	100	12.39	32.89	18.03	-	-	-	-	-	-	-	-
3-Octanone*	7.101	128	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20.53	-	-	-	-	-	-	-	-

## Supplementary information

[illegible]