Supplementary Materials:

Effects of Carbon, Nitrogen, Ambient pH and Light on Mycelial Growth, Sporulation, Sorbicillinoid Biosynthesis and Related Gene Expression in *Ustilaginoidea virens*

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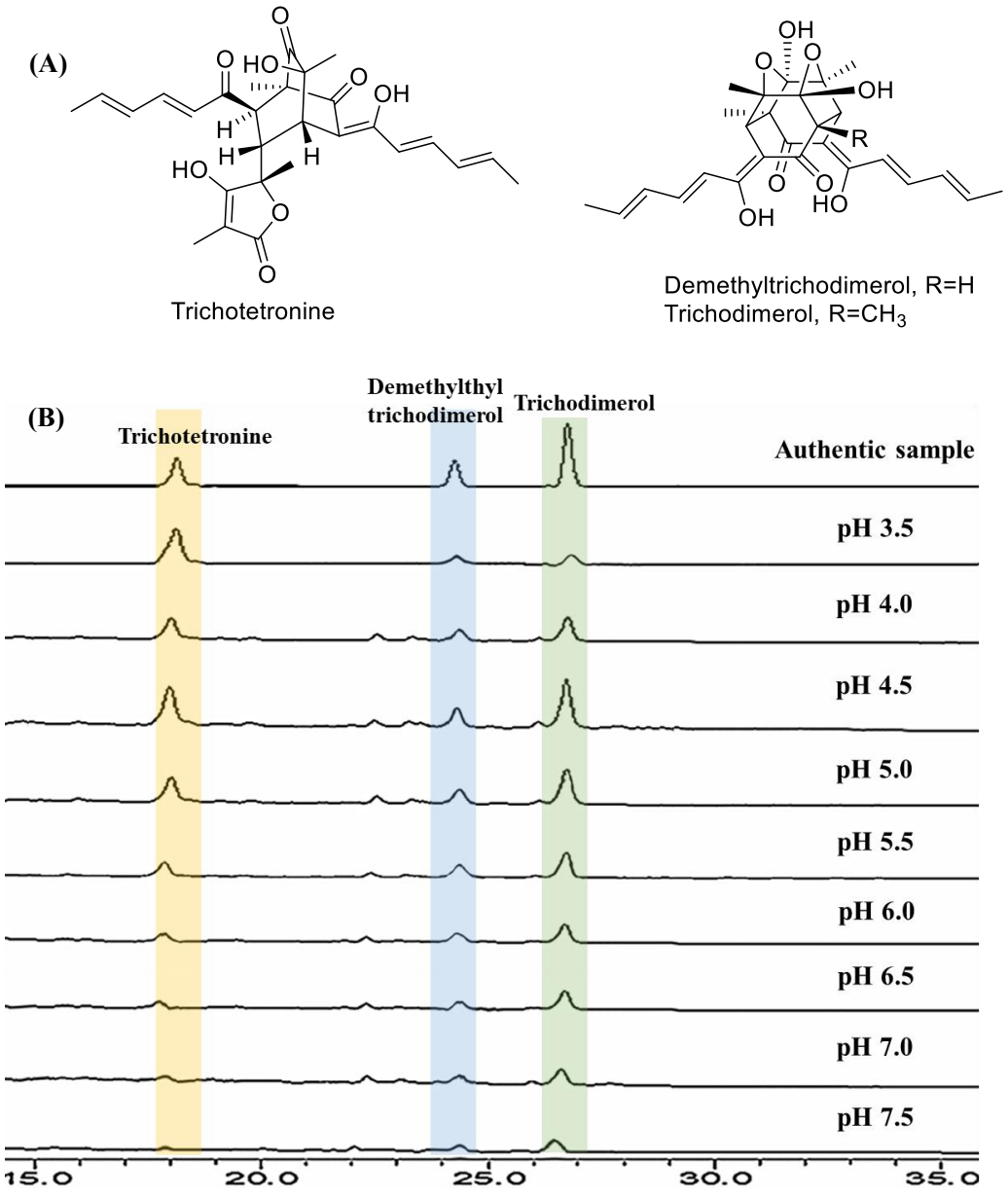

Figure S1. Effects of ambient pH on the production of main sorbicillinoids in *U. virens*. (**A**) Chemical structures of trichotetronine, demethylthyltrichodimerol and trichodimerol; (**B**) HPLC profiles of the *U. virens* cultivated in PBGY medium with different ambient pH values.

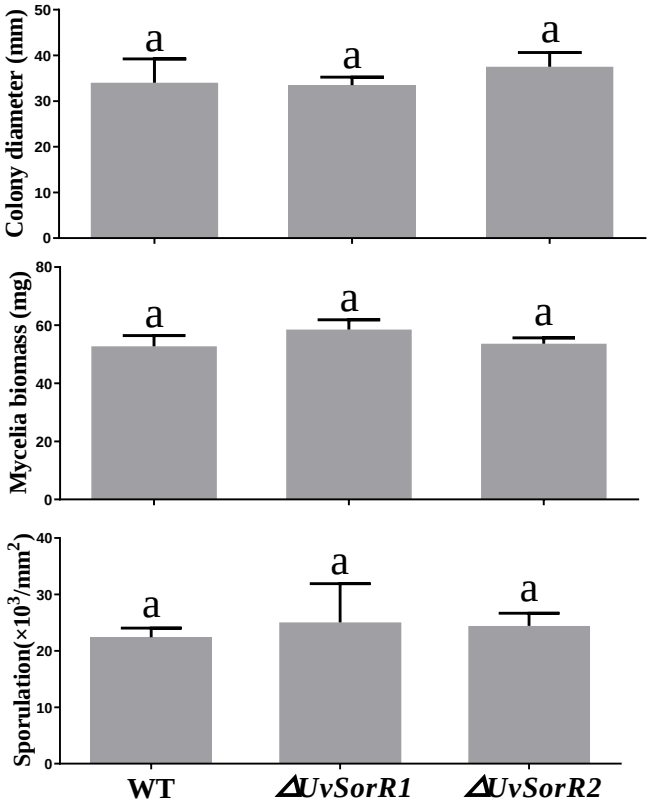

Figure S2. Comparisons of the colony extension, mycelial growth and sporulation of wild-type (WT) strain P1 and gene deletion mutants (Δ*UvSorR1* and Δ*UvSorR2*) of *U. virens* cultured on PDA medium at 28 °C for 18 days. The colony diameter (mm), mycelial biomass (mg) and sporulation (conidia/mm²) were measured to statistical analysis. The error bars represent the standard deviations, and the asterisks represent significant differences at $p < 0.05$. All data showed that Δ*UvSorR1* and Δ*UvSorR2* mutants had no significant differences with WT strain.

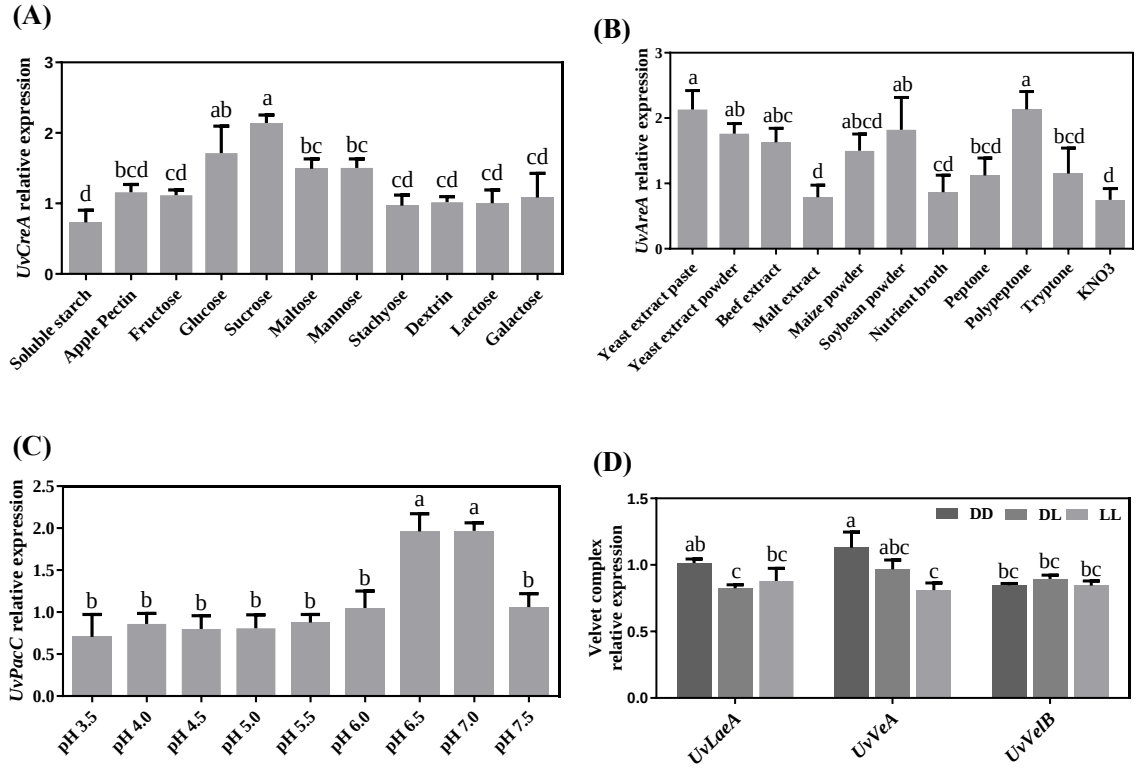

Figure S3. Expressions of global regulator genes in *U. virens* grown on different carbon and nitrogen sources, ambient pH, and light treatments. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method, and normalized using the *β-tubulin* as housekeeping gene. (**A**) Relative expression analysis of *UvCreA* gene under the various carbon sources; (**B**) Relative expression analysis of *UvAreA* gene under the various nitrogen sources; (**C**) Relative expression analysis of *UvPacC* gene under different ambient pH; (**D**) Relative expression analysis of velvet complex (*UvLaeA*, *UvVeA*, and *UvVelB*) under different light exposure treatments. Different letters in each figure mean significant difference according to Duncan's Multiple Range Test ($p < 0.05$).

Table S1. Fungal strains and plasmids used in this study.

Fungal Strain/Plasmid		Description	Ref.
Fungal strain	*Ustilaginoidea virens* P1	Wild-type	[57]
	Δ*UvSorR1*	*UvSorR1* deletion mutant of wild type P1	This study
	Δ*UvSorR2*	*UvSorR2* deletion mutant of wild type P1	This study
Plasmid	pCas9-tRp-gRNA	Cas9-gRNA vector with the tRNA promoter	[58]
	pFL2	pFL2 containing geneticin-resistance (GenR)	[71]
	pCas9-tRp-UvSorR1	Cas9-gRNA vector with the UvSorR1 spacer	This study
	pCas9-tRp-UvSorR2	Cas9-gRNA vector with the UvSorR2 spacer	This study

Note: This table is supplemental to the experimental procedure.

Table S2. Primers used in this study.

Primer	Oligonucleotide Sequence (5'-3')	Uses
UvSorR1_5F	CGGACCTCGTCATCTTCAT	*UvSorR1*'5 flanks amplification
UvSorR1_5R	CAGATACGGCAGAGAAATCGCAACCTCA AGAGCGAGACATTCAGGGA	
UvSorR1_3F	GTTTAGATTCCAAGTGTCTACTGCTGGCG GAAGAACAACACGACCAT	*UvSorR1*' 3 flanks amplification
UvSorR1_3R	ACGGCAAGATGACAGACAG	
UvSorR1 _F	GCTACGCTTGGCATCCTTG	*UvSorR1* transformant screening
UvSorR1 _R	CGTATTAGCAACCCGAGACC	
KOUvSorR1_5F	TGATGATGAGGCGGTGAAG	*UvSorR1* transformants 5F and 3F screening
KOUvSorR1_3R	TAGCCCATCCTGCCTGACT	
UvSorR1_spacer_F	ACCTAAGAAGGAGAGATCTGACCG	Constructing pCas9-tRp-UvSorR1 vector
UvSorR1_spacer_R	AAACCGGTCAGATCTCTCCTTCTT	
UvSorR2_5F	GAACGCTCGGTTTCCTGTG	*UvSorR2*'5 flanks amplification
UvSorR2_5R	CAGATACGGCAGAGAAATCGCAACCTCG AACGGCAAATCGCAGTAG	
UvSorR2_3F	GTTTAGATTCCAAGTGTCTACTGCTGGCA TGGAGTTACGCCCAGGAG	*UvSorR2*'3 flanks amplification
UvSorR2_3R	GCTGTGCTGAACGCTCTTG	
UvSorR2_F	CACCATCACAGCCACGAAT	*UvSorR2* transformant screening
UvSorR2_R	GCAATGTGGAGGTAATAGACG	
KOUvSorR2_5F	AGTGTCGCAATGGTGGTGAT	*UvSorR2* transformants 5F and 3F screening
KOUvSorR2_3R	TCAGTTCGTCGTAGGTGGC	
UvSorR2_spacer_F	ACCTATATCGCGATCCGTACCGAG	Constructing pCas9-tRp-UvSorR2 vector
UvSorR2_spacer_R	AAACCTCGGTACGGATCGCGATAT	
GEN_F	GAGGTTGCGATTTCTCTGCCGTATCTG	*GenR* amplication from pFL2
GEN_R	GCCAGCAGTAGACACTTGGAATCTAAAC	
855_R	TGTTGGGTTTGAGCTAGGTGGG	Upstream /downstream flanks amplification
856_F	GAATGGTCAAATCAAACTGCTAGATAT	
β-tubulin _F	AGGTTGCGTTGAAGGAGGTT	*U. virens β-tubulin* as the control
β-tubulin _R	GAGGTGGAGTTGCCGATAAA	
RT_UvSorA_F	CTTTTCGACGCGGCATTCTT	RT-qPCR for *UvSorA*
RT_UvSorA_R	GCACAAAGACGCCAGTGAAG	
RT_UvSorB_F	GGACCTCCATGTGGTTCCAG	RT-qPCR for *UvSorB*
RT_UvSorB_R	TGATAGCGCTCTTCCCAACG	
RT_UvSorC_F	CGTCTCTGTCCACGTCCTTG	RT-qPCR for *UvSorC*
RT_UvSorC_R	CAGAGGCGTTGGCTGTTTTG	
RT_UvSorD_F	CCATCCACGTTACCCCGATT	RT-qPCR for *UvSorD*
RT_UvSorD_R	CCATGAGCCCTCCATCTTGG	

RT_UvSorR1_F	CAGGGCCTCCACCAAATTCT	RT-qPCR for *UvSorR1*
RT_UvSorR1_R	AGGTGGCTGTTCTCGTCAAG	
RT_UvSorR2_F	GCGGTCGACACAGGAAGATT	RT-PqCR for *UvSorR2*
RT_UvSorR2_R	CGCTGTGGCAGTGCAATTAG	
RT_ UvSorT_F	GGTCTATTGTGTAGCGCCGT	RT-qPCR for *UvSorT*
RT_ UvSorT_R	GAACCGGTCCGTATGTCTCG	
RT-UvLaeA_F	CTGCGAGGTCTGTGGTGAAT	RT-qPCR for *UvLaeA*
RT-UvLaeA_R	CTACCTCCCAACGTGCGATT	
RT-UvVeA_F	GAGTAGTTACGCTGGGGCTC	RT-qPCR for *UvVeA*
RT-UvVeA_R	CCGTACATCAAGTCGGAGCA	
RT-UvVelB_F	TGTAGCATTTGCGTGCGATG	RT-qPCR for *UvVelB*
RT-UvVelB_R	ACCCAGCCGTGATTCAACAT	
RT-UvPacC _F	CAAGATTACGTCCGGTGCCT	RT-qPCR for *UvPacC*
RT-UvPacC _R	AGTTCCACTGGCACGTAAGG	
RT-UvAreA_F	ATTCCCATCAAGCCTCGCAA	RT-qPCR for *UvAreA*
RT-UvAreA_R	CCAGCTGTGTTGCTGTTGAC	
RT-UvCreA_F	GTGAATGGAGGAGGGGCATC	RT-qPCR for *UvCreA*
RT-UvCreA_R	CCCTCCTCACCCACCTTTTC	

Note: This table is supplemental to the experimental procedure.

Table S3. Effects of carbon sources on contents and yields of main sorbicillinoids (trichotetronine, demethylthyltrichodimerol, trichodimerol) in *U. virens.*

Carbon Source	Trichotetronine		Demethylthyltrichodimerol		Trichodimerol		Total Sorbicillinoids	
	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield(mg/L)
Soluble starch	0.0884±0.0047 g	0.0440±0.0036 e	0.0000±0.0000 h	0.0000±0.0000 f	0.0202±0.0060 d	0.0101±0.0033 g	0.1086±0.0106 f	0.0541±0.0068 e
Apple pectin	1.5433±0.0491 c	2.2503±0.3322 d	0.1257±0.0149 d	0.1820±0.0211 c	0.1263±0.0153 c	0.1824±0.0143 e	1.7952±0.0594 c	2.6147±0.3532 d
Fructose	3.6631±0.2403 a	5.0723±0.5539 b	0.3163±0.0134 b	0.4377±0.0373 b	1.0997±0.0840 a	1.5167±0.0504 a	5.0792±0.1789 a	7.0267±0.5415 a
Glucose	1.8514±0.0524 b	5.8074±0.2287 a	0.0496±0.0095 e	0.1555±0.0293 c	0.2243±0.0218 b	0.7045±0.0821 c	2.1253±0.0768 b	6.6674±0.3243 a
Sucrose	1.4384±0.0775 cd	4.7949±0.4608 bc	0.0444±0.0032 ef	0.1476±0.0088 c	0.1064±0.0043 c	0.3539±0.0127 d	1.5892±0.0795 d	5.2963±0.4791 c
Maltose	1.3233±0.0547 de	4.5765±0.1734 c	0.1534±0.0083 c	0.5310±0.0390 a	0.2284±0.0230 b	0.7880±0.0417 b	1.7050±0.0748 cd	5.8954±0.1863 b
Mannose	0.5929±0.0416 f	1.8978±0.0314 d	0.0187±0.0011 g	0.0602±0.0071 d	0.1201±0.0088 c	0.3848±0.0250 d	0.7317±0.0447 e	2.3429±0.0126 d
Stachyose	0.1372±0.0202 g	0.1999±0.0111 e	0.0395±0.0026 ef	0.0582±0.0070 d	0.0323±0.0023 d	0.0476±0.0067 fg	0.2090±0.0240 f	0.3056±0.0219 e
Dextrin	0.1260±0.0206 g	0.1963±0.0086 e	0.0320±0.0068 fg	0.0510±0.0160 de	0.0563±0.0018 d	0.0888±0.0106 f	0.2143±0.0184 f	0.3361±0.0190 e
Lactose	0.1452±0.0280 g	0.0823±0.0045 e	0.0000±0.0000 h	0.0000±0.0000 f	0.0202±0.0017 c	0.0116±0.0015 g	0.1654±0.0286 f	0.0939±0.0031 e
Galactose	1.2190±0.0669 e	0.2680±0.0144 e	0.3759±0.0126 a	0.0826±0.0010 d	0.1053±0.0115 d	0.0232±0.0028 g	1.7003±0.0817 cd	0.3738±0.0177 e

Note: All values represent mean ± standard deviation; values marked with different letters in each column indicate significant differences ($p < 0.05$).

Table S4. Effects of nitrogen sources on contents and yields of main sorbicillinoids (trichotetronine, demethylthyltrichodimerol, trichodimerol) in *U. virens*.

Nitrogen Source	Trichotetronine		Demethylthyltrichodimerol		Trichodimerol		Total Sorbicillinoids	
	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield(mg/L)
Yeast extract	1.4102 ±0.1417 a	7.4642 ±1.1144 a	0.2954 ±0.0198 b	0.5634 ±0.0513 b	0.2954 ±0.0198 cd	1.5552 ±0.0221 c	1.8127 ±0.1412 a	9.5828 ±1.1479 a
Yeast extract powder	0.0260 ±0.0013 h	0.1415 ±0.0077 e	0.0105 ±0.0010 e	0.0571 ±0.0051 g	0.0244 ±0.0043 g	0.1326 ±0.0224 f	0.0610 ±0.0060 g	0.3312 ±0.0308 f
Beef extract	1.1222 ±0.0827 b	4.9952 ±0.2952 b	0.1734 ±0.0105 a	0.7718 ±0.0209 a	0.3338 ±0.0154 bc	1.4861 ±0.0389 c	1.6294 ±0.1019 b	7.2531 ±0.3196 b
Malt extract powder	0.5785 ±0.0727 de	2.0152 ±0.1082 d	0.0356 ±0.0035 d	0.1242 ±0.0036 de	0.1099 ±0.0083 e	0.3836 ±0.0028 e	0.7240 ±0.0844 d	2.5230 ±0.1133 e
Maize powder	0.6586 ±0.0147 d	2.1525 ±0.0192 d	0.0330 ±0.0034 d	0.1079 ±0.0080 ef	0.2499 ±0.0156 d	0.8166 ±0.0414 d	0.9415 ±0.0293 c	3.0770 ±0.0346 de
Soybean powder	0.5404 ±0.0226 def	1.9873 ±0.0332 d	0.0413 ±0.0059 cd	0.1517 ±0.0195 de	0.1062 ±0.0066 e	0.3903 ±0.0138 e	0.6879 ±0.0324 de	2.5293 ±0.0504 e
Nutrient broth	0.4531 ±0.0267 f	2.3228 ±0.4001 d	0.0150 ±0.0021 e	0.0760 ±0.0028 fg	0.0961 ±0.0071 ef	0.4895 ±0.0553 e	0.5642 ±0.0332 e	2.8883 ±0.4578 de
Peptone	0.9858 ±0.0182 c	5.7209 ±0.0996 b	0.0504 ±0.0059 c	0.2925 ±0.0336 c	0.5516 ±0.0180 a	3.2016 ±0.1219 a	1.5878 ±0.0209 b	9.2150 ±0.1521 a
Poly peptone	0.4643 ±0.0456 ef	2.4848 ±0.3151 d	0.0308 ±0.0074 d	0.1619 ±0.0177 d	0.1442 ±0.0129 e	0.7728 ±0.1059 d	0.6392 ±0.0642 de	3.4195 ±0.4210 d
Tryptone	0.6018 ±0.0310 d	3.7598 ±0.6001 c	0.0437 ±0.0065 cd	0.2689 ±0.0153 c	0.3556 ±0.0855 b	2.1761 ±0.3363 b	1.0010 ±0.0734 c	6.2048 ±0.3780 c
KNO_3	0.3008 ±0.0109 g	0.8939 ±0.0124 e	0.0146 ±0.0033 e	0.0433 ±0.0094 g	0.0462 ±0.0027 fg	0.1374 ±0.0122 f	0.3615 ±0.0112 f	1.0745 ±0.0209 f

Note: All values represent mean ± standard deviation; values marked with different letters in each column indicate significant differences ($p < 0.05$).

Table S5. Effects of ambient pH on contents and yields of main sorbicillinoids (trichotetronine, demethylthyltrichodimerol, trichodimerol) in *U. virens*.

Ambient pH	Trichotetronine		Demethylthyltrichodimerol		Trichodimerol		Total Sorbicillinoids	
	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield(mg/L)
pH 3.5	1.8940 ±0.2754 a	3.3235 ±1.0248 a	0.0193 ±0.0033 c	0.0327 ±0.0042 d	0.0756 ±0.0050 e	0.1345 ±0.0499 f	1.9889 ±0.2721 a	3.4907 ±1.0718 bc
pH 4.0	1.4207 ±0.2375 b	3.4230 ±0.8297 b	0.0643 ±0.0100 a	0.1529 ±0.0180 cd	0.2675 ±0.0297 b	0.6367 ±0.0484 de	1.7525 ±0.2084 ab	4.2126 ±0.8146 bc
pH 4.5	1.0746 ±0.0253 c	5.7990 ±0.3861 c	0.0528 ±0.0050 ab	0.2853 ±0.0361 bc	0.4101 ±0.0153 a	2.2105 ±0.0913 a	1.5375 ±0.0338 b	8.2949 ±0.4921 a
pH 5.0	0.8891 ±0.0274 c	6.4376 ±0.2469 a	0.0477 ±0.0067 ab	0.3455 ±0.0523 ab	0.2008 ±0.0174 c	1.4543 ±0.1422 b	1.1375 ±0.0277 c	8.2374 ±0.3194 a
pH 5.5	0.4322 ±0.1116 d	3.1214 ±0.7718 b	0.0562 ±0.0283 ab	0.4162 ±0.2246 ab	0.1185 ±0.0276 d	0.8546 ±0.1796 c	0.6069 ±0.0858 d	4.3922 ±0.6022 b
pH 6.0	0.2483 ±0.0402 de	1.9917 ±0.1714 c	0.0606 ±0.0083 ab	0.4865 ±0.0283 a	0.0827 ±0.0117 e	0.6722 ±0.1323 cd	0.3916 ±0.0381 de	3.1504 ±0.0512 cd
pH 6.5	0.1919 ±0.0075 def	1.4139 ±0.0823 c	0.0422 ±0.0026 b	0.3111 ±0.0264 bc	0.0619 ±0.0017 e	0.4561 ±0.0082 e	0.2960 ±0.0085 ef	2.1811 ±0.1066 d
pH 7.0	0.0774 ±0.0064 e	0.5007 ±0.0401 d	0.0159 ±0.0013 c	0.1029 ±0.0084 d	0.0283 ±0.0024 f	0.1833 ±0.0159 f	0.1217 ±0.0078 fg	0.7869 ±0.0484 e
pH 7.5	0.0255 ±0.0038 e	0.0680 ±0.0087 d	0.0098 ±0.0002 c	0.0261 ±0.0012 d	0.0118 ±0.0013 f	0.0315 ±0.0039 f	0.0471 ±0.0051 g	0.1257 ±0.0123 e

Note: All values represent mean ± standard deviation; values marked with different letters in each column indicate significant differences ($p < 0.05$).

Table S6. Effects of light exposure on contents and yields of main sorbicillinoids (trichotetronine, demethylthyltrichodimerol, trichodimerol) in *U. virens*.

Light	Trichotetronine		Demethylthyltrichodimerol		Trichodimerol		Total Sorbicillinoids	
	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield (mg/L)	Content (mg/g)	Yield(mg/L)
DD	0.9162±0.0638 c	4.9952±0.2952 a	0.1416±0.0076 b	0.7718±0.0209 b	0.2540±0.0427 c	1.3831±0.2109 a	1.3118±0.1085 c	7.1501±0.4864 ab
DL	1.6071±0.3253 b	5.8267±0.9588 a	0.2996±0.0112 a	1.0911±0.0214 a	0.3692±0.0669 b	1.3382±0.1743 a	2.2759±0.4002 b	8.2560±1.1168 a
LL	3.7917±0.4554 a	4.9648±0.1285 a	0.3681±0.0722 a	0.4829±0.0786 c	0.9557±0.0488 a	1.2616±0.1442 a	5.1154±0.5027 a	6.7093±0.1320 b

Note: DD, 24 h darkness/day; LL, 24 h light/day; DL,12 h darkness-12 h light/day: All values represent mean ± standard deviation. The values marked with different letters in each column indicate significant differences ($p < 0.05$).