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1 **Supporting Information**

2 **S1 Text. Preparation of the HB-4 Medium Solution**

3 The HB-4 medium containing (per liter of distilled water): 0.2 g $(NH_4)_2SO_4$, 1 mL saturated
4 solution of $Ca(H_2PO_4)_2 \cdot H_2O \cdot CaSO_4$, 0.08 g $MgSO_4 \cdot 7H_2O$, 0.1 g $NaHCO_3$, 0.025 g KCl, 0.15 mL
5 1% $FeCl_3$-solution, 0.5 mL soil extract. The pH was adjusted within the range 7 to 7.2 using NaOH or
6 HCl. The medium solution was sterilized at 121℃ for 15 min. Its validity period is 2 months.

7 **S2 Text. Protein Sample Extraction**

8 The treated algae cells were collected and centrifuged at 10,000 rpm for 10 min. The resulting pellet
9 was washed twice with phosphate buffer (5.0 mmol L^{-1}, pH 7.8). The algae cells were frozen and
10 homogenized in a pre-chilled mortar using a pestle. Four millilitres of phosphate buffer (5.0 mmol
11 L^{-1}, pH 7.8) was added during the homogenization. The homogenate was then centrifuged at 10,000
12 rpm for 10 min at 4 °C. The supernatant was stored at -80 °C. Bovine serum albumin was used as a
13 standard when determining the total soluble protein concentration.

14 **S3 Text. Method of Algae Cell Ultrastructural Observation by TEM**

15 Cells collected by centrifugation at 3000 rpm for 15 min were fixed in 0.1 M cacodylate buffer
16 including 2.5% glutaraldehyde for more than 24 h. The cells were then embedded in agar and washed
17 three times with phosphate buffer. They were post-fixed with 1% osmium tetroxide in 0.1 M phosphate
18 buffer, dehydrated in an ethanol series and embedded in Spurr resin. Ultrathin sections were then cut
19 with a diamond knife and stained with uranyl acetate and lead citrate. Later, they were examined using
20 an H-7500 electron microscope at the Institute of Agro-products Processing of Chinese Academy of
21 Agricultural Sciences.

22 **S4 Text. Sample Extraction and Chemical Analysis**

23 Two portions of 40-mL treated algae samples were harvested at each experimental time (1, 2, 3, 4,

24 5, 6 and 7 d), and all of them were centrifuged at 4000 rpm for 5 min. The fresh weight (FW) of the
25 resulting algae biomass was immediately recorded. The supernatant and control media without algae
26 were analysed directly on the HPLC-MS/MS. One portion of the cell samples were added to 5 mL
27 ethyl acetate, sufficiently shaken with a vortex, ultrasonicated for 15 min to damage the cell wall and
28 facilitate the extraction and then centrifuged at 4000 rpm for 5 min. The extract was filtered through 2
29 g of anhydrous sodium sulphate for dehydration and transferred to a pear-shaped flask. The remaining
30 part was re-extracted with the same extraction method, and the combined extract was evaporated on a
31 vacuumed rotary evaporator at 35 °C. The residues in the flask were dissolved with mobile phase
32 (acetonitrile) for the determination of epoxiconazole enantiomers by HPLC-MS/MS. Another portion
33 of cell samples was resuspended for 3 min in 5 mL of fresh culture solution and then centrifuged at
34 4000 rpm for 5 min. The supernatant was discarded to remove the fungicide adsorbed on cell surfaces,
35 and the residues were extracted and determined using the method described above.

36 The HPLC-MS/MS analysis method in reported in our previous study. [1] In brief, the liquid
37 chromatograph was an Agilent 1200 HPLC system equipped with a G1322A degasser, a G1311A
38 quaternary pump, a G1316B column compartment, a G1329A autosampler and a 100-µL sample loop.
39 A new highly efficient chiral column [Phenomenex LuxCellulose-1 column, 250 × 4.6 mm (i.d.)] was
40 used for the stereoselective separation. A mixture of acetonitrile/water/formic acid (95:5:0.1, v/v/v)
41 was used as the mobile phase at a flow rate of 1 mL min^{-1}. The injection volume was maintained at 5
42 µL, and the optical rotation detection wavelength was 426 nm. Chromatographic separation was
43 conducted at a temperature of 30 °C. The first eluted enantiomer was (−)-epoxiconazole, and the
44 second was (+)-epoxiconazole. An Agilent 6460 triple-quadrupole mass spectrometer equipped with
45 an electrospray ionization source was used (Agilent, USA) for MS/MS analysis. Data acquisition and
46 processing was performed using Masshunter (Agilent, USA) software. The analyses were performed
47 in the positive ion (ESI+) mode. The capillary, fragmentor, collision energies, dry gas temperature and

48 flow rate were 3500 V, 110 V, 10 V, 14 V, 350 °C and 11 L h^{-1}, respectively. The transition of precursor
49 ion (m/z 330.1) to epoxiconazole (m/z 121 and 141) was detected with multiple reaction monitoring
50 (MRM). The ion m/z 121 was used for quantification. The nebulizer gas was 99.95% nitrogen with a
51 pressure of 40 psi, and the collision gas was 99.999% nitrogen.

52 Work standard solutions of epoxiconazole for calibration curves were prepared using
53 epoxiconazole stock solutions diluted by matrix solutions from blank samples (algae cells or culture
54 medium) or acetonitrile. The linear regression equations and the correlation coefficients for each
55 enantiomer were obtained from the peak area ratios plotted against the respective concentrations (0.1
56 to 1 mg L^{-1}). The external matrix-matched standards were used to eliminate the matrix effect and obtain
57 more accurate results in algae and medium. The standard curves for (−)- and (+)-enantiomers showed
58 excellent linearity with $R^2 > 0.99$ in the range of 0.1−1 mg L^{-1} of each enantiomer (n = 3).

59 A series of blank samples (algae cells or culture medium) fortified with *rac*-epoxiconazole at 0.1,
60 0.5 and 1 mg kg^{-1} were determined immediately after fortification. The recovery rates and their
61 standard errors were acceptable. The LOQs and LODs for both enantiomers in algae or culture medium
62 were found to be 0.01 and 0.003 mg kg^{-1}, respectively. The LOQ was defined as the lowest spiking
63 level of each stereoisomer at acceptable recovery and precision.

64 **Reference**

65 1 C. Liu, B. Wang, P. Xu, C. Cheng, J. Diao, Z. Q. Zhou. *Journal of Agricultural and Food Chemistry*,
66 2014, 62, 360-367.

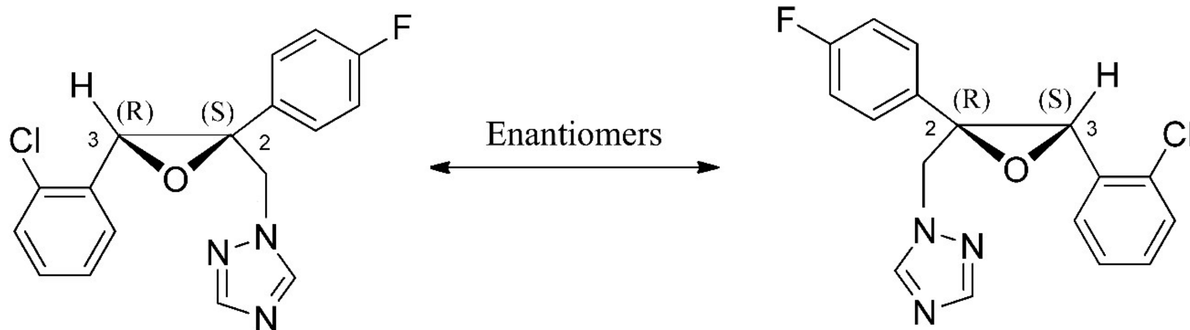

Fig. S1. Structures of the present commercial product of epoxiconazole enantiomers.

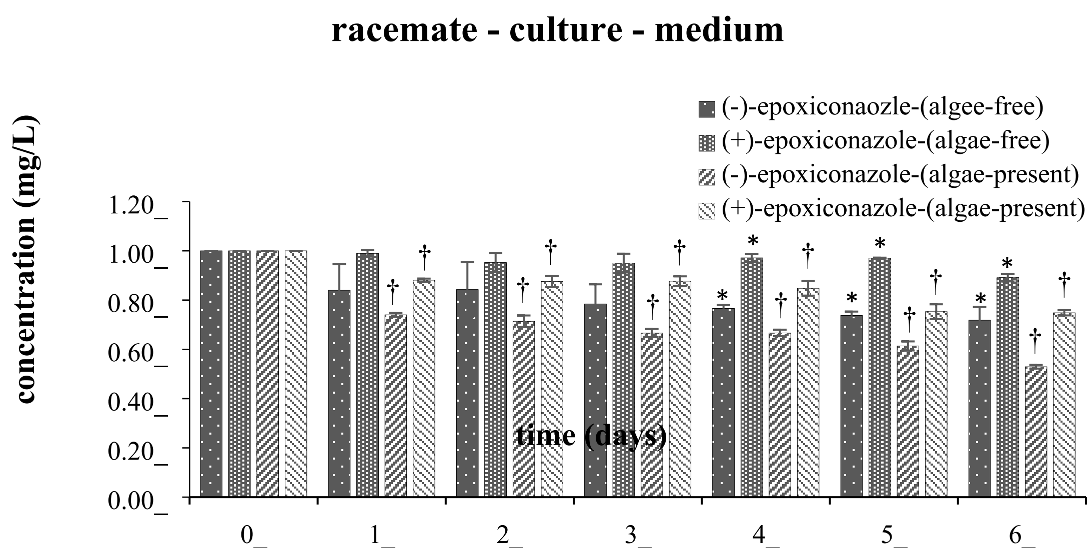

Fig. S2. Dissipation of epoxiconazole enantiomers in medium when treated with rac-epoxiconazole (bars are standard error). * and † indicate significant difference between the two enantiomers in the absence and presence of algae, respectively, at the same time point (S-N-K test, $P < 0.05$).

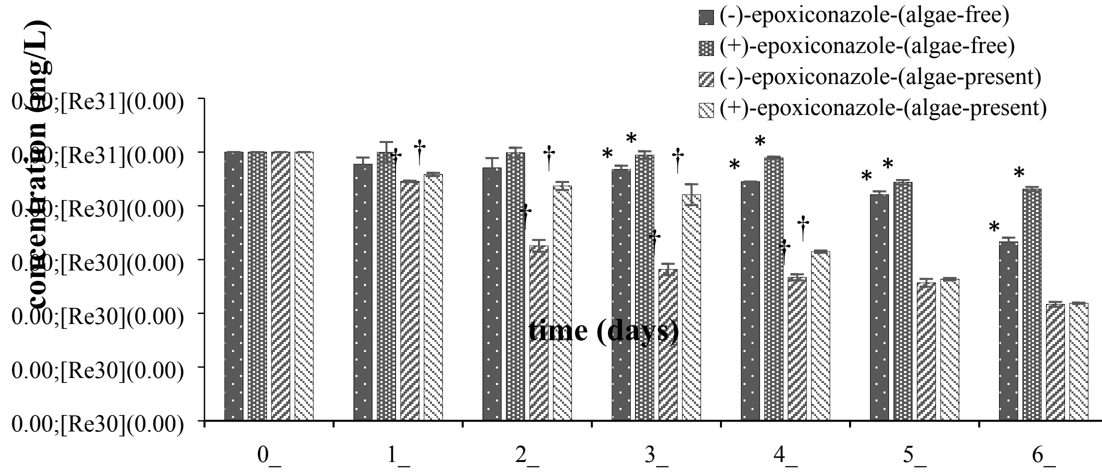

Fig. S3. Dissipation of epoxiconazole enantiomers in medium when treated with (-)- or (+)-epoxiconazole (bars are standard error). ∗ and † indicate significant difference between the two enantiomers in the absence and presence of algae, respectively, at the same time point (S-N-K test, $P < 0.05$).