Journal of Plant Interactions

ISSN: 1742-9145 (Print) 1742-9153 (Online) Journal homepage: https://www.tandfonline.com/loi/tjpi20

Chaetominine, (+)-alantrypinone, questin, isorhodoptilometrin, and 4-hydroxybenzaldehyde produced by the endophytic fungus *Aspergillus* sp. YL-6 inhibit wheat (*Triticum aestivum*) and radish (*Raphanus sativus*) germination

Gui Ren-Yi, Xu Lei, Kuang Yi, Chung III-Ming, Qin Jian-Chun, Liu Li, Yang Sheng-Xiang & Zhao Li-Chun

To cite this article: Gui Ren-Yi, Xu Lei, Kuang Yi, Chung III-Ming, Qin Jian-Chun, Liu Li, Yang Sheng-Xiang & Zhao Li-Chun (2015) Chaetominine, (+)-alantrypinone, questin, isorhodoptilometrin, and 4-hydroxybenzaldehyde produced by the endophytic fungus *Aspergillus* sp. YL-6 inhibit wheat (*Triticum aestivum*) and radish (*Raphanus sativus*) germination, Journal of Plant Interactions, 10:1, 87-92, DOI: 10.1080/17429145.2015.1019742

To link to this article: https://doi.org/10.1080/17429145.2015.1019742

Published online: 12 Mar 2015.

Journal of Plant Interactions, 2015
Vol. 10, No. 1, 87–92, http://dx.doi.org/10.1080/17429145.2015.1019742

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Chaetominine, (+)-alantrypinone, questin, isorhodoptilometrin, and 4-hydroxybenzaldehyde produced by the endophytic fungus *Aspergillus* sp. YL-6 inhibit wheat (*Triticum aestivum*) and radish (*Raphanus sativus*) germination

Gui Ren-Yi[a,b], Xu Lei[a,b], Kuang Yi[a,b], Chung III-Ming[c], Qin Jian-Chun[d], Liu Li[a,b], Yang Sheng-Xiang[a,b,c]* and Zhao Li-Chun[e]

[a]*The Nurturing Station for the State Key Laboratory of Subtropical Silviculture, Zhejiang A & F University, Lin'an, Zhejiang Province 311300, China;* [b]*Key Laboratory of Chemical Utilization of Forestry Biomass of Zhejiang Province, Zhejiang A & F University, Lin'an, Zhejiang 311300, China;* [c]*Department of Applied Bioscience, Konkuk University, Seoul 143-701, Republic of Korea;* [d]*College of Plant Science, Jilin University, Changchun, Jilin 130062, China;* [e]*Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine, Nanning, Guangxi 53001, China*

(*Received 29 October 2014; accepted 25 January 2015*)

Five phytotoxic substances with allelopathic activity were isolated from endophytic fungi *Aspergillus* sp. YL-6 habited in *Pleioblastus amarus*. The chemical structures of these substances were determined as chaetominine (**1**), (+)-alantrypinone (**2**), questin (**3**), isorhodoptilometrin (**4**), and 4-hydroxybenzaldehyde (**5**) by nuclear magnetic resonance and mass spectrometry data. The potential allelopathic effects of compounds **1–5** were evaluated on wheat (*Triticum aestivum*) and radish (*Raphanus sativus*). Under lab condition, at concentrations of 10 and 20 ppm, compounds **1–5** inhibit the germination and growth of the two tested seeds completely. An idole-3-acetic acid (IAA) derivative, (+)-alantrypinone (**2**) displayed the best inhibitory effects on radish seeds among these tested compounds with the similar activity as the positive control glyphosate, a broad-spectrum systemic herbicide. In the further evaluation of compounds **1–5**, Questin (**3**), an anthraquinone derivative, can inhibit shoot and root elongation of wheat, the inhibitory effects assessed were similar to the positive control glyphosate.

Keywords: *Pleioblastus amarus*; *Aspergillus* sp. YL-6; herbicides; allelochemicals

Introduction

Allelopathy is a process by which plants, including microorganisms, produce special chemicals that can be either beneficial or harmful to other (or even their own) neighboring organisms (Hiroshi et al. 2014). *Pleioblastus amarus* is widely distributed in southern China and is used in traditional medicine for the treatment of inflammation, fever, and the prevention of senility (Sun et al. 2014). Some biological activity experiments have shown that *P. amarus* leaf extracts have remarkable immunomodulatory properties (Wang et al. 2004). Interestingly, little weeds grow around *P. amarus* and this plant rarely suffers from diseases and insect pests. The possible reason of these interesting phenomena might be attributed to the allelochemicals which are produced by the plant directly or by its endophytic fungi.

Endophytic fungi produce a large number of bioactive products possessing structural diversities such as alkaloids, steroids, terpenoids, isocoumarin derivatives, quinones, phenols, and lactones (Verma et al. 2009). Furthermore, the plethora of substances is seemingly produced as part of a defense strategy, as well as contributing to various physiological functions of the host plants (Strobel et al. 2004). Because of the ecological roles of endophytes, the chemical components in the endophytic fungi become attractive and sustainable for agricultural pharmacology. In our ongoing research of *P. amarus*, we are interested to explore the endophytic fungi and their potential contribution on the defense strategy of their host plant.

In our previous assay, we found a strain from *P. amarus*, named *Aspergillus* sp. YL-6, exhibited inhibitory effects on seeds growth. The aim of this study, therefore, was to determine the allelochemicals present in the plant endophytic fungi *Aspergillus* sp. YL-6 associated with *P. amarus* on the basis of a bioassay-guided fractionation. Five active compounds were obtained and their structures were identified to be chaetominine (**1**), (+)-alantrypinone (**2**), questin (**3**), isorhodoptilometrin (**4**), and 4-hydroxybenzaldehyde (**5**) by nuclear magnetic resonance (NMR) and mass spectrometry (MS) data.

Materials and methods

General procedure

NMR spectra were recorded on a Bruker AVANCE III (500 MHz) instrument. Chemical shifts were calculated using solvent residual as the internal standard. Electrospray ionization mass spectrometry was conducted with a Thermo LCQ Fleet instrument. Column chromatography (CC) was performed on silica gel (90−150 μm;

*Corresponding author. Email: shengxiangyang2000@163.com
Ren-Yi and Lei contributed equally to this study.

Qingdao Marine Chemical Inc., Qingdao, China), MCI gel (75−150 μm; Mitsubishi Chemical Corp., Tokyo, Japan), Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (40−63 μm; Merck, Darmstadt, Germany). GF254 plates (Qingdao Marine Chemical Inc.) were used for thin-layer chromatography (TLC).

Plant samples

Stem tissues of *P. amarus* were collected from Zhejiang A & F University. Samples were brought to the laboratory in separate sterile polythene bags and processed within four hours of collection.

Fungal material

The samples were rinsed gently in running tap water to remove dusts and debris. The stem was cut into segments (0.5–1 cm). The samples were dipped in 75% ethanol for 1 minute, immersed in 4% sodium hypochlorite for 3 minutes and then rinsed in sterile distilled water for 30 s. The sterilized tissue samples were plated on potato dextrose agar (PDA) medium contained in Petri dishes, amended with chloramphenicol (150 mg/L). The Petri dishes were sealed using parafilm and incubated at 26°C at 12-h light/dark (L/D) cycle. The Petri dishes were monitored every day to check the growth of endophytic fungal colonies from the segments.

The hyphal tips which grew out from the segments were isolated and subcultured on PDA medium. The pure cultures were maintained on PDA slants. The researched endophytic fungus *Aspergillus* sp. YL-6 was identified by Prof. Mao Feng-Sheng (Zhejiang A & F University) according to its macroscopic and microscopic characteristics such as the morphology of fruiting structures and spore morphology.

Cultivation

After growing on PDA medium at 28°C for seven days, the fungus was inoculated in liquid medium containing: $CaCl_2$ 0.5 g, KH_2PO_4 0.1 g, KCl 0.05 g, $MgSO_4{\cdot}7H_2O$ 0.1 g, glucose 20.0 g, peptone 15.0 g in 1000 ml H_2O. The pH was adjusted to 6.0 before autoclaving. Fermentation was carried out in 1000 ml flasks each containing 200 ml medium on a rotary shaker at 150 rpm at 28°C for seven days.

Extraction and isolation

The culture broth (30 L) of *Aspergillus* sp. YL-6 was filtered to obtain the mycelium and water phase. The mycelium was dried at 50°C, and smashed directly with ultrasonic extraction by ethyl acetate and followed by acetone for three times. These two parts were combined and dried after TLC, then defatted with cyclohexane after being dissolved in methanol to get a crude extract (6.2 g). The crude extract was then subjected to a silica gel column and eluted with a chloroform/MeOH mixture (100:1, 50:1, and 20:1) to give three fractions (Fr.1–Fr.3). Fr.1 was further purified on Sephadex LH-20, silica gel, and preparative TLC to afford **2** (11 mg). Fr.2 was further chromatographed by CC on RP-18, Sephadex LH-20, silica gel, and prepared TLC to yield compounds **1** (8.2 mg) and **5** (6.8 mg). Fr.3 was rechromatographed on a Sephadex LH-20 column, and further purified by prepared TLC to give compounds **3** (12 mg) and **4** (9.5 mg).

*Chaetominine (**1**)*

White powder. ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 171.8 (C-10), 165.3 (C-17), 159.8 (C-15), 147.3 (C-24), 146.6 (C-23), 138.6 (C-9), 136.6 (C-4), 134.5 (C-21), 129.7 (C-7), 127.1 (C-20), 127.0 (C-22), 126.3 (C-19), 125.3 (C-6), 124.8 (C-5), 121.0 (C-18), 114.2 (C-8), 82.3 (C-2), 76.3 (C-3), 59.4 (C-11), 49.9 (C-14), 38.1 (C-13), 13.9 (C-12). ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 8.29 (1H, *brs*, H-24), 8.19 (1H, *brd*, *J* = 7.5 Hz, H-19), 7.87 (1H, *td*, *J* = 7.5, 1.5 Hz, H-21), 7.70 (1H, *brd*, *J* = 8.0 Hz, H-22), 7.59 (1H, *brd*, *J* = 7.5 Hz, H-20), 7.52 (1H, *brd*, *J* = 8.0 Hz, H-8), 7.50 (1H, *brd*, *J* = 7.0 Hz, H-5), 7.44 (1H, *7d*, *J* = 7.5, 1.0 Hz, H-7), 7.26 (1H, *td*, *J* = 7.5, 1.0 Hz, H-6), 6.72 (1H, *d*, H-OH-3), 5.93 (1H, *brs*, H-14), 5.62 (1H, *s*, H-2), 4.62 (1H, *q*, *J* = 7.0 Hz, H-11), 1.61(1H, *d*, *J* = 6.5 Hz, H-12), 2.55 (1H, *dd*, *J* = 13.0, 3.0 Hz, H-13*α*), 2.94 (1H, *t*, *J* = 13.0 Hz, H-13*β*).

*(+)-Alantrypinone (**2**)*

White powder. ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 169.6 (C-1), 159.8 (C-12), 154.8 (C-4), 147.1 (C-6), 135.3 (C-18), 135.2 (C-20), 134.5 (C-8), 127.9 (C-7, C-9), 127.8 (C-25), 126.8 (C-10), 122.8(C-22), 120.6 (C-11), 119.8 (C-23), 118.6 (C-24), 112.2 (C-21), 106.0 (C-17), 55.1 (C-3), 54.6 (C-14), 26.1 (C-15), 18.8 (C-16). ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 11.22 (1H, *s*, 19-NH), 9.68 (1H, *s*, 2-NH), 8.15 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-10), 7.82 (1H, *ddd*, *J* = 7.5, 7.0, 1.5 Hz, H-8), 7.65 (1H, *d*, *J* = 8.0 Hz, H-7), 7.54 (1H, *ddd*, *J* = 8.5, 7.0, 1.5 Hz, H-9), 7.41 (1H, *d*, *J* = 8.0 Hz, H-24), 7.38 (1H, *d*, *J* = 8.0 Hz, H-21), 7.13 (1H, *ddd*, *J* = 8.0, 7.0, 1.5 Hz, H-22), 7.00 (1H, *ddd*, *J* = 8.0, 7.0, 1.5 Hz, H-23), 5.71(1H, *t*, *J* = 3.0 Hz), 3.44 (1H, *dd*, *J* = 17.0, 2.5 Hz, H-15*α*), 3.24 (1H, *dd*, *J* = 17.0, 2.5 Hz, H-15*β*), 2.13 (3H, *s*, H-16).

*Questin (**3**)*

Yellow powder. ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 186.9 (C-9), 182.6 (C-10), 164.6 (C-6), 163.9 (C-8), 162.2 (C-1), 146.7 (C-3), 137.3 (C-11), 132.5 (C-14), 124.6 (C-2), 119.6 (C-4), 114.9 (C-13), 112.5 (C-12), 107.5 (C-5), 105.5 (C-7), 56.8 (C-8-OMe), 21.8 (C-3-Me). ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 13.26 (1H, *s*, H-1-OH), 11.19 (1H, *brs*, H-3-OH), 7.45 (1H, *d*, *J* = 1.5 Hz, H-4), 7.22 (1H, *d*, *J* = 2.0 Hz, H-5), 7.14 (1H, *d*, *J* = 1.5 Hz, H-2), 6.86 (1H, *d*, *J* = 2.0 Hz, H-7), 3.92 (3H, *s*, H-8-OMe), 2.40 (3H, *s*, H-3-Me).

Isorhodoptilometrin (4)

Yellow powder. ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 190.1 (C-9), 182.0 (C-10), 164.9 (C-8), 163.2 (C-6), 161.6 (C-1), 150.5 (C-3), 135.7 (C-10a), 133.1 (C-4a), 125.3 (C-2), 121.5 (C-4), 114.2 (1a), 112.8 (9a), 109.3 (C-5), 108.4 (C-7), 67.0 (C-2′), 45.6 (C-1′), 23.9 (C-3'). ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 7.57 (1H, *d*, *J* = 1.5 Hz, H-4), 7.21 (1H, *d*, *J* = 1.5 Hz, H-2), 7.14 (1H, *d*, *J* = 2.5 Hz, H-5), 6.61 (1H, *d*, *J* = 2.5 Hz, H-7), 3.91 (1H, *m*, H-2′), 2.74 (2H, *m*, H-1′), 1.11 (3H, *d*, *J* = 6.5 Hz, H-3′).

4-Hydroxybenzaldehyde (5)

White powder. ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 191.4 (C-1), 163.8 (C-5), 132.6 (C-3 and C-7), 128.9 (C-2), 116.3 (C-4 and C-5). ^{1}H NMR (500 MHz, DMSO-d_6) δ_H: 10.61 (1H, *brs*, H-5-OH), 9.79 (1H, *s*, H-1-CHO), 7.76 (2H, *d*, *J* = 9.0 Hz, H-3 and H-7), 6.94 (2H, *d*, *J* = 8.5 Hz, H-4 and H-6).

Seed germination and seedling growth bioassay

The seeds of two herbaceous plants, wheat (*Triticum aestivum*) and radish (*Raphanus sativus*), were used for the allelopathic bioassay. The procedures were conducted according to the reported protocol (Kuang et al. 2014). The plant seeds were imbibed in deionized water for two hours, soaked in 0.3% $KMnO_4$ for 15 minutes, and washed with sterile water until they were colorless. The compounds, positive control and blank solvent acetone, were placed in a 9-cm Petri dish lined with a Whatman No. 1 filter paper to final concentrations of 1.25, 2.5, 5, 10, and 20 ppm. After the evaporation of acetone, the plant seeds were sown in the Petri dish and irrigated with deionized water. Three repeats were conducted. The Petri dishes were placed in an illuminated growth chamber held at 25 ± 1°C, 80 ± 2% RH and a 12/12 h L/D photoperiod for 90 hours.

The germination rates were calculated according to Equation 1. Allelopathic effects (response index [RI]) were calculated according to Equation 2 (Zhang et al. 2013).

$$\text{Germination rate (\%)} = \frac{\text{(number of germinated seeds)}}{\text{(total number of seeds)}} \quad (1)$$

$$\begin{aligned} &\text{If } T > C, \text{then RI} = 1 - C/T; \\ &\text{if } T < C, \text{then RI} = T/C - 1 \end{aligned} \quad (2)$$

where *T* is the length of the treatment, *C* is the length of the blank control, and RI is the RI. The RI values range from +1 to −1, with positive values indicating stimulation and negative values indicating inhibition relative to the controls. The RI was considered to be superior to the T/C statistical method and easy to interpret because it is simply the proportional reduction of the treatment relative to the control (Bruce & Richardson 1998).

Germination rates and RIs were expressed as means ± standard deviation (SD) for three replicates. All of the statistical differences were compared by SPSS software using one-way analysis of variance at an $\alpha \leq 0.05$ significance level.

Results and discussion

Identification of allelochemicals

Chemical investigations of *Aspergillus* sp. YL-6 crude extracts led to the isolation of five compounds (Figure 1) by multiple chromatographic procedures: chaetominine (**1**) (Jiao et al. 2006), (+)-alantrypinone (**2**) (Larsen et al. 1998), questin (**3**) (Kimura et al. 1983), isorhodoptilometrin (**4**) (Ren et al. 2006), and 4-hydroxybenzaldehyde (**5**) (Liu et al. 2009). Their structures were identified on the basis of comparison of their NMR and MS data with those reported compounds.

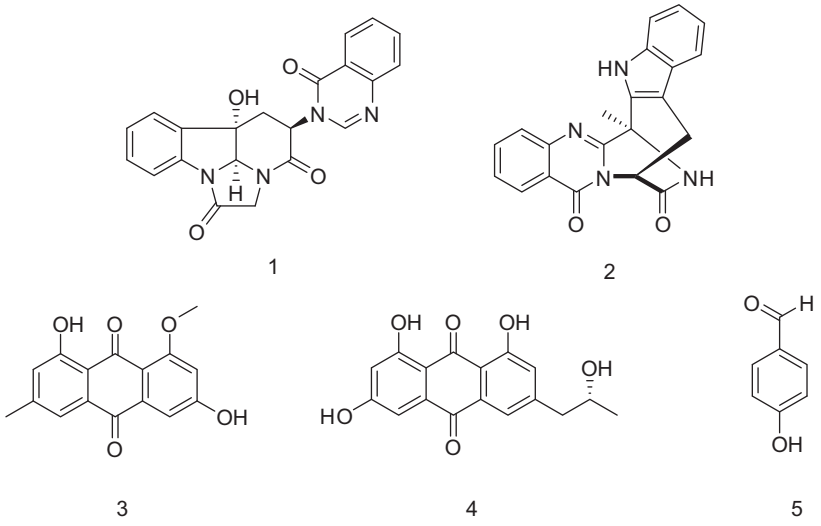

Figure 1. Structures of compounds **1–5** (Chaetominine (**1**), (+)−Alantrypinone (**2**), Questin (**3**), Isorhodoptilometrin (**4**), and 4-Hydroxybenzaldehyde (**5**)).

Allelopathic activity

All isolated compounds were evaluated for allelopathic activity against radish (*R. sativus*) and wheat (*T. aestivum*) seeds by determining the germination rates and seedling growth (root and shoot elongation) with respect to the control, glyphosate, a broad-spectrum systemic herbicide.

As shown in Table 1, all the isolated compounds inhibit the germination of radish (*R. sativus*) in a dose-dependent manner. The inhibition increased with increasing concentrations of these compounds **1–5**, when the concentrations up to 10 ppm all the tested compounds inhibited the germination of radish completely. Compared with glyphosate, compounds **2–5** showed a significant higher inhibition on the germination of radish at the concentrations from 1.25 ppm to 5.0 ppm. Compounds **2–5** can inhibit the elongation of radish roots (RI values ranging from −0.20 to −0.35 at 1.25 to 5 ppm) with the similar activity as the positive control glyphosate (RI values ranging from −0.02 to −0.21 at 1.25 to 5 ppm). The similar inhibitory effects were also found on the shoot elongation of radish, compounds **2, 3** and **5** did not show significant difference compared with glyphosate from the concentration 1.25 ppm to 5 ppm. The inhibition increased with increasing concentrations of these compounds. The root and shoot growth of the tested weeds were suppressed completely at the concentration of 10 ppm and 20 ppm. The results indicate that the compounds **2, 3** and **5** exhibit similar inhibitory effects on radish as the positive control glyphosate at lower concentration of 5 ppm.

Interestingly, compound **2,** that has an indol fragment in its structure, possessed strong phytotoxic activity on the seeds germination in our results. An idole-3-acetic acid (IAA), a common plant growth regulator, also has an indol fragment in its structure. The indol fragment in **2** maybe play an important role in the allelopathic assays. And because of the similar structure systems of IAA and compound **2**, the latter might act as IAA mechanism in the plant growth regulation.

For a further evaluation of compounds **1–5**, the inhibitory effects of wheat were also assessed. As shown in Table 2, on seed germination, compounds **1–5** exhibit similar effects as glyphosate at the lower concentration of 5 ppm. When the concentration up to 10 ppm all the tested compounds could completely inhibit the germination of wheat seed. The positive control glyphosate exhibits the best inhibitory effects among all the tested compounds on the root elongation of wheat at a lower concentration of 2.5 ppm. Compounds **3** and **5** show the similar inhibitory activity of the root elongation of wheat (RI = −0.42 and −0.34) as glyphosate (RI = −0.38) at the concentration of 5 ppm. For the inhibition on shoot elongation of wheat, compounds **1**, **2**, **4**, and **5** did not produce significant inhibitory activity at the concentration lower than 5 ppm. While for the observed inhibition by **3** of wheat shoot elongation, compound **3** was found to exhibit similar inhibitory effects (RI values ranging from −0.53 to −0.75 at 1.25 to 5 ppm) as our positive

Table 1. Allelopathic effects on radish (*R. sativus*) of compounds **1–5**[a].

	Germination rate				Root elongation (RI)				Shoot elongation (RI)			
	1.25 ppm	2.5 ppm	5 ppm	10 ppm	20 ppm	1.25 ppm	2.5 ppm	5 ppm	10 ppm	20 ppm	1.25 ppm	2.5 ppm	5 ppm	10 ppm	20 ppm
1	0.92 ± 0.08 a	0.82 ± 0.12 a	0.38 ± 0.06 bc	0 ± 0 c	0 ± 0 c	0.04 ± 0.03 a	−0.31 ± 0.06 c	−1 ± 0 c	−1 ± 0 b	−1 ± 0 b	−0.02 ± 0.06 ab	−0.19 ± 0.05 bc	−1 ± 0 b	−1 ± 0 b	−1 ± 0 b
2	0.58 ± 0.15 bc	0.43 ± 0.06 c	0.08 ± 0.06 cd	0 ± 0 c	0 ± 0 c	−0.20 ± 0.06 b	−0.18 ± 0.08 abc	−0.08 ± 0.05 a	−1 ± 0 b	−1 ± 0 b	−0.15 ± 0.01 bc	−0.13 ± 0.01 ab	−0.47 ± 0.07 a	−1 ± 0 b	−1 ± 0 b
3	0.65 ± 0.13 bc	0.63 ± 0.21 b	0.40 ± 0.25 b	0 ± 0 c	0 ± 0 c	0.01 ± 0.01 a	−0.03 ± 0.03 a	−0.11 ± 0.08 a	−1 ± 0 b	−1 ± 0 b	−0.20 ± 0.07 c	−0.24 ± 0.05 bc	−0.43 ± 0.02 a	−1 ± 0 b	−1 ± 0 b
4	0.50 ± 0.13 c	0.52 ± 0.10 bc	0.23 ± 0.08 bcd	0 ± 0 c	0 ± 0 c	−0.07 ± 0.08 ab	−0.25 ± 0.07 bc	−0.35 ± 0.05 b	−1 ± 0 b	−1 ± 0 b	0.02 ± 0.02 a	0 ± 0.04 a	−0.48 ± 0.10 a	−1 ± 0 b	−1 ± 0 b
5	0.72 ± 0.06 b	0.55 ± 0.15 bc	0.45 ± 0.13 b	0 ± 0 c	0 ± 0 c	−0.13 ± 0.07 ab	−0.12 ± 0.09 ab	−0.30 ± 0.03 b	−1 ± 0 b	−1 ± 0 b	−0.40 ± 0.06 d	−0.34 ± 0.07 c	−0.47 ± 0.04 a	−1 ± 0 b	−1 ± 0 b
gp	0.92 ± 0.03 a	0.85 ± 0.09 a	0.80 ± 0.05 a	0.75 ± 0.05 b	0.73 ± 0.08 b	−0.02 ± 0.05 ab	−0.10 ± 0.01 ab	−0.21 ± 0.07 ab	−0.21 ± 0.07 a	−0.45 ± 0.01 a	−0.19 ± 0.04 c	−0.26 ± 0.02 bc	−0.33 ± 0.03 a	−0.13 ± 0.04 a	−0.16 ± 0.12 a
ck	0.95 ± 0 a	0.95 ± 0 a	0.95 ± 0 a	0.95 ± 0 a	0.95 ± 0 a										

Note: gp standards for glyphosate and ck for the blank control.
[a]Means (±SD) within columns followed by the same letter are not significantly different at $p \leq 0.05$.

Table 2. Allelopathic effects on wheat (*T. aestivum*) of compounds **1–5**[a].

	Germination rate					Root elongation (RI)					Shoot elongation (RI)				
	1.25 ppm	2.5 ppm	5 ppm	10 ppm	20 ppm	1.25 ppm	2.5 ppm	5 ppm	10 ppm	20 ppm	1.25 ppm	2.5 ppm	5 ppm	10 ppm	20 ppm
1	0.93 ± 0.05 b	0.88 ± 0.07 bc	0.90 ± 0.05 abc	0 ± 0 c	0 ± 0 c	−0.18 ± 0.07 c	−0.19 ± 0.04 b	−0.19 ± 0.08 b	−1 ± 0 b	−1 ± 0 b	−0.08 ± 0.01 b	−0.07 ± 0.03 a	−0.15 ± 0.06 a	−1 ± 0 b	−1 ± 0 b
2	0.95 ± 0.05 ab	0.90 ± 0.10 abc	0.83 ± 0.12 c	0 ± 0 c	0 ± 0 c	0.18 ± 0.02 b	0.28 ± 0.02 a	0.09 ± 0.05 a	−1 ± 0 b	−1 ± 0 b	0.01 ± 0.03 ab	−0.01 ± 0.07 a	−0.14 ± 0.06 a	−1 ± 0 b	−1 ± 0 b
3	1 ± 0 a	0.98 ± 0.03 ab	0.85 ± 0.05 bc	0 ± 0 c	0 ± 0 c	−0.15 ± 0.03 c	−0.16 ± 0.06 b	−0.42 ± 0.02 c	−1 ± 0 b	−1 ± 0 b	−0.53 ± 0.02 c	−0.63 ± 0.01 b	−0.75 ± 0.02 b	−1 ± 0 b	−1 ± 0 b
4	0.93 ± 0.07 b	0.87 ± 0.10 c	0.85 ± 0.05 bc	0 ± 0 c	0 ± 0 c	0.21 ± 0.03 ab	0.27 ± 0.05 a	0.03 ± 0.04 a	−1 ± 0 b	−1 ± 0 b	0.08 ± 0.02 a	−0.04 ± 0.04 a	−0.17 ± 0.08 a	−1 ± 0 b	−1 ± 0 b
5	0.98 ± 0.02 ab	0.85 ± 0.05 c	0.70 ± 0.18 d	0 ± 0 c	0 ± 0 c	0.31 ± 0.01 a	0.17 ± 0.01 a	−0.34 ± 0.05 bc	−1 ± 0 b	−1 ± 0 b	−0.07 ± 0.07 b	−0.10 ± 0.07 a	−0.30 ± 0.06 a	−1 ± 0 b	−1 ± 0 b
gp	1 ± 0 a	0.93 ± 0.06 abc	0.97 ± 0.05 ab	0.93 ± 0.05 b	0.93 ± 0.05 b	−0.28 ± 0.04 d	−0.37 ± 0.02 c	−0.38 ± 0.02 c	−0.8 ± 0.02 a	−0.73 ± 0.01 a	−0.47 ± 0.02 c	−0.56 ± 0.05 b	−0.58 ± 0.06 b	−0.93 ± 0.01 a	−0.95 ± 0.01 a
ck	1 ± 0 a	1 ± 0 a	1 ± 0 a	1 ± 0 a	1 ± 0 a										

Note: gp standards for glyphosate and ck for the blank control.
[a]Means (±SD) within columns followed by the same letter are not significantly different at $p \leq 0.05$.

control glyphosate (RI values ranging from −0.47 to −0.58 at 1.25 to 5 ppm), which was stronger than the other compounds (RI values ranging from −0.08 to −0.30 at 1.25 to 5 ppm). In particular, at a lower concentration of 1.25 ppm, the RI of **3** was still up to −0.53. Our observations suggest that **3** could play an important role in plant growth regulation.

Questin (**3**), an anthraquinone derivative, was first found in a typical fungi *Penicillium frequentans* (Mahmoodian & Stickings 1964). Later it was discovered in many plants and microorganisms with various bioactivities, such as antimicrobial activities (Inamori et al. 1983; Du et al. 2014). Some antimicrobial activities of **3** may directly or indirectly affect the growth of other plants. Al Saadawi et al. (1983) pointed out that some organisms produce allelopathic chemicals that inhibited soil bacteria and changed the soil environment to their own advantage (Al Saadawi et al. 1983). Moreover, emodin, an isomer of **3** differs for the position of methoxyl, also exhibited good phytotoxic activity on weed growth (Inoue et al. 1992), which means that the methoxylation of the hydroxyl on the aromatic ring would not be a necessary procedure for the weed growth inhibition activity. Further studies will be carried out to investigate the inhibition mechanism and ecological relevance of allelopathy with respect to the effective presence and persistence time.

Funding

This work was supported by the Program for Key Science and Technology Team of Zhejiang Province [grant number 2013TD17]; the Talent Foundation of Zhejiang A & F [grant number 2014FR066]; the National Natural Science Foundation of China [grant number 31200262], [grant number 31000149], [grant number 31470414]; the Ku research professor program, Konkuk University.

References

Al Saadawi IS, Rice EL, Karns TKB. 1983. Allelopathic effects of *Polygonum aviculare* L. III. Isolation, characterization, and biological activities of phytotoxin other than phenol. J Chem Ecol. 9:761–774.

Bruce WG, Richardson D. 1988. Bioassays for allelopathy: measuring treatment responses with independent controls. J Chem Ecol. 14:181–187.

Du FY, Li XM, Song JY, Li CS, Wang BG. 2014. Anthraquinone derivatives and an orsellinic acid ester from the marine alga-derived endophytic fungus *Eurotium cristatum* EN-220. Helve Chim Acta. 97:973–978.

Hiroshi A, Yosuke N, Junnosuke O. 2014. Isolation of (2*S*,4*R*)-2-amino-4-methyl-hex-5-enoic acid, a nonprotein amino acid, as an allelochemical from the fruiting bodies of *Boletus fraternus* Peck. J Plant Interact. 9:627–631.

Inamori Y, Kato Y, Kubo M, Kamiki T, Takemoto T, Nomoto K. 1983. Studies on metabolites produced by *Aspergillus terreus* var. *aureus*. I. Chemical structures and antimicrobial activities of metabolites isolated from culture broth. Chem Pharm Bull. 31:4543–4548.

Inoue M, Nishimura H, Li HH, Mizutani J. 1992. Allelochemicals from *Polygonum sachalinense* Fr. Schm (Polygonaceze). J Chem Ecol. 18:1883–1840.

Jiao RH, Xu S, Liu JY, Ge HM, Ding H, Xu C, Zhu HL, Tan RX. 2006. Chaetominine, a cytotoxic alkaloid produced

by endophytic *Chaetomium* sp. IFB-E015. Org Lett. 8:5709–5712.

Kimura Y, Kozawa M, Baba K, Hata K. 1983. New constituents of roots of *Polygonum cuspidatum*. Planta Med. 48:164–168.

Kuang Y, Liang YK, Yang SX, Liu L, Wang ZK. 2014. Allelopathic effects of *Pleioblastus kongosanensis* f. aureostriaus on germination and growth of *Vigna radiate*. Allelopathy J. 33:77–82.

Larsen TO, Frydenvang K, Frisvad JC, Christophersen C. 1998. UV-guided isolation of alantrypinone, a novel *Penicillium alkaloid*. J Nat Prod. 61:1154–1157.

Liu Y, Jiang JH, Zhang Y, Chen YG. 2009. Chemical constituents of *Dendrobium aurantiacum* var. denneanum. Chem Nat Compd. 45:525–527.

Mahmoodian A, Stickings CE. 1964. The biochemistry of microorganisms. CXV. Metabolites of *Penicillium frequentans*; isolation of sulochrin, asterric acid, (+)–bisdechlorogeodin and two new substituted anthraquinones, questin and questinol. Biochem J. 92:369–378.

Ren H, Tian L, Gu GQ, Zhu WM. 2006. Secalonic acid D; A cytotoxic constituent from marine lichen-derived fungus *Gliocladium* sp. T3. Arch Pharm Res. 29:59–63.

Strobel G, Daisy B, Castillo U, Harper J. 2004. Natural products from endophytic microorganisms. J Nat Prod. 67:257–268.

Sun J, Zhang P, Wei Q, Xun H, Tang F, Yue YD, Li L, Guo XF, Zhang R. 2014. Amarusine A, a new dioxaspiro[4.4] nonane derivative with a butyrolactone ring from *Pleioblastus amarus*. Tetrahedron Lett. 55:4529–4513.

Verma VC, Kharwar RN, Strobel GA. 2009. Chemical and functional diversity of natural products from plant associated endophytic fungi. Nat Prod Commun. 4:1511–1532.

Wang HB, Yao H, Bao GH, Zhang HP, Qin GW. 2004. Flavone glucosides with immunomodulatory activity from the leaves of *Pleioblastus amarus*. Phytochem. 65:969–974.

Zhang Q, Wang SQ, Tang HY, Li XJ, Zhang L, Xiao J, Gao YQ, Zhang AL, Gao JM. 2013. Potential allelopathic indole diketopiperazines produced by the plant endophytic *Aspergillus fumigatus* using the one strain–many compounds method. J Agr Food Chem. 61:11447–11452.