Bull Environ Contam Toxicol (2016) 96:235–241
DOI 10.1007/s00128-015-1676-4

Effects of Tricyclazole on Cadmium Tolerance and Accumulation Characteristics of a Dark Septate Endophyte (DSE), *Exophiala pisciphila*

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Received: 16 June 2015 / Accepted: 6 October 2015 / Published online: 14 October 2015

Abstract *Exophiala pisciphila* is a cadmium-tolerant fungus, and produces 1,8-dihydroxynaphthalene melanin which can be inhibited by tricyclazole. Tricyclazole at higher levels (20 and 40 μg mL^{-1}) reduced the growth and sporulation of *E. pisciphila*, but toxicity was not observed at a low concentration (2.5 μg mL^{-1}). Under cadmium (Cd) stress (50, 100 and 200 mg L^{-1}), 2.5 μg mL^{-1} of tricyclazole reduced fungal growth and sporulation. These reduces indicated a decrease on Cd tolerance of *E. pisciphila*. For both the 0 and 2.5 μg mL^{-1} tricyclazole treatments, Cd was associated mostly with cell walls and was extracted by 2 % acetic acid and 1 M NaCl. The FTIR spectra of the *E. pisciphila* mycelia were similar for both 0 and 2.5 μg mL^{-1} tricyclazole treatments, which showed hydroxyl, amine, carboxyl and phosphate groups. Thus inhibition of melanin synthesis by tricyclazole did not change Cd accumulation characteristics in *E. pisciphila*. Results suggested that melanin played a protective role for *E. pisciphila* against Cd stress, but inhibition of melanin synthesis did not have a remarkable impact on Cd accumulation in *E. pisciphila*.

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Keywords Tricyclazole · Cadmium · *Exophiala pisciphila* · Growth · Accumulation characteristics

Melanized fungi are commonly found in soils of different ecosystems, especially in harsh environments (van der Wal et al. 2009). Fungal melanin is considered to be an important protective factor against environmental stresses such as UV radiation, high concentrations of salts, heavy metals, and radionuclides (Gessler et al. 2014). The protective role of melanins for fungi against heavy metals is of increasing concern due to the massive use and dispersion into soils of heavy metals (Fomina and Gadd 2003; Loayza-Muro et al. 2012; McLean et al. 1998). Melanins are considered to be important for melanized fungi to survive in environments polluted by heavy metals (Haferburg and Kothe 2007).

Dark septate endophytes (DSE) are a group of ascomycetes dominant in plant roots (Jumpponen 2001; Jumpponen and Trappe 1998) and are commonly found in heavy metal polluted soils (Deram et al. 2008; Li et al. 2012; Ruotsalainen et al. 2007; Zhang et al. 2008, 2013). The dark-pigmented hyphae of DSE indicate high levels of melanins in the mycelia and 1,8-dihydroxynaphthalene (DHN) melanin was shown to exist in the mycelia of a DSE (Zhan et al. 2011). Some studies found that the contents of melanin in DSE mycelia increased markedly with increased concentrations of Cd and Pb in the media (Ban et al. 2012; Zhan et al. 2011). However, whether melanins play an important role in DSE tolerance to heavy metals stress has not been determined.

Tricyclazole (5-methyl-1,2,4-triazolo[3,4-b]benzothiazole), a specific inhibitor of 1,8-dihydroxynaphthalene (DHN) melanin synthesis, can very effectively block melanin synthesis and does not cause toxic effects at very low doses (Kogej et al. 2004;

Lee et al. 2003). For determining the functions of DHN melanins, it is useful to use tricyclazole at nontoxic concentrations to inhibit DHN melanin synthesis and then analyze the changes in fungal tolerance to environmental stresses (Bell and Wheeler 1986). In this way, we can determine the role of melanin in the tolerance of DSE to heavy metals.

In the present study, the influence of tricyclazole at different concentrations (0–40 μg mL^{-1}) on the growth and sporulation of a DSE, *Exophiala pisciphila*, was investigated to first select a nontoxic concentration. We then investigated the effects of DHN melanin synthesis inhibition by tricyclazole at nontoxic concentrations on the cadmium (Cd) tolerance, accumulation features and Fourier transform infrared spectroscopy (FTIR) of *E. pisciphila*. We hypothesized that melanins play a certain role in the tolerance *E. pisciphila* to Cd stress. The inhibition of melanin synthesis by tricyclazole has some impacts on Cd tolerance and the accumulation characteristics of *E. pisciphila*.

Materials and Methods

The DSE fungus *Exophiala pisciphila* was isolated from an abandoned lead–zinc mining area in Huize County, Yunnan Province, China, and was preserved in the Agricultural Culture Collection of China encoded as ACCC32496. The strain was routinely maintained on potato glucose agar slants and stored at 4°C in refrigerator. A subculture was made every 2 months. MMN medium ($CaCl_2{\cdot}2H_2O$, 0.05 g L^{-1}; NaCl, 0.025 g L^{-1}; glucose 10.0 g L^{-1}; maltose 3.0 g L^{-1}; NaCl 0.025 g L^{-1}; K_2HPO_4 0.5 g L^{-1}; VB_1, 0.1 mg L^{-1}; $MgSO_4{\cdot}7H_2O$, 0.15 g L^{-1}; 1 % $FeCl_3$ solution 1.2 mL L^{-1}; $NaNO_3$, 3.0 g L^{-1}; pH 5.5) was used for culturing the inoculums (Zhan et al. 2011).

The treatments with tricyclazole concentrations of 0, 2.5, 5.0, 7.5, 10, 20 and 40 μg mL^{-1} in MMN medium were obtained by adding appropriate amounts of a stock solution of tricyclazole. According to the EC_{50} value (111.2 mg L^{-1}) of *E. pisciphila* to Cd (Zhan et al. 2015), the treatments with Cd concentrations of 50, 100 and 200 mg L^{-1} in MMN were obtained by adding appropriate amounts of a stock solution of $CdCl_2{\cdot}2.5H_2O$ to the medium to obtain mean measured Cd concentrations of 46.6, 97.8 and 189.5 mg L^{-1} after medium sterilization. These concentrations represented 93.2 %–97.8 % of the nominal concentrations (Table 1).

Fungal colonies with a diameter of 6 mm were taken as inoculums with a sterilized punch from the actively growing *E. pisciphila* plates and the colonies were inoculated into different culture plates and flasks in sextuplet. The inoculated plates were cultured at 28 ± 1°C for 35 days. From the 9th day, the fungal colony's diameter was measured every 2 days and the area calculated (cm^2). The inoculated flasks were cultured at 28 ± 1°C and shaken at 120 rpm for 7 days. The fungal mycelia were first collected, moisture was removed by suction filtration and then baked at 70–75°C for at least 48 h, and finally, the dry weight of the mycelia was measured. The number of spores was analyzed using a hemacytometer.

Dry mycelial samples (0.20 g for each sample) were mineralized by wet digestion in 15 mL of an ultrapure mixture of concentrated $HNO_3/HClO_4$ (3:1) (v/v) on a thermo block at 200–250°C to obtain a transparent solution and then diluted with 0.2 % HNO_3 to 50 mL. The total concentrations of Cd were determined by flame atomic absorption spectrometry (AAS) using a TAS-990 atomic absorption spectrometer (Beijing Puxi Instrument Factory, Beijing, P.R. China). Quartz sand was a standard material for assurance control.

The mycelia in liquid media were isolated by filtration, washed three times with distilled water, and suction filtered by vacuum to obtain fresh mycelia. Fresh mycelia were accurately weighed to 0.50 g and homogenized in extraction buffer (0.25 mol L^{-1} sucrose, Tris–HCl buffer solution (pH 7.5) and 1.0 mmol L^{-1} DL-Dithioery thritol) according to Weigel and Jager (1980), with some modifications (Wang et al. 2008). In brief, the homogenate was centrifuged at 600 rpm for 15 min and the precipitate was designated as the "cell wall fraction" consisting mainly of cell walls and cell wall debris. The resulting supernatant solution was further centrifuged at 16,000 rpm for 45 min. The resultant deposit and supernatant solution were referred to as the "cell organelles fraction" and "soluble fraction," respectively. All steps were performed at 4°C. The fractions were dried and wet digested separately and the Cd concentrations in the digests then determined by flame atomic absorption spectrometry (AAS, model TAS990).

Cd associated with different chemical forms was successively extracted by designated solutions in the following order (Yang et al. 1995): (1) 80 % ethanol, extracting inorganic Cd including nitrate, chloride, and aminophenol cadmium; (2) deionized water, extracting soluble Cd-organic acid complexes and $Cd(H_2PO_4)_2$; (3) 1 M NaCl, extracting Cd integrated with pectates and protein; (4) 2 % acetic acid (HAc), extracting insoluble $CdHPO_4$, $Cd_3(PO_4)_2$ and other Cd–phosphate complexes; (5) 0.6 M HCl, extracting cadmium oxalate; and (6) Cd in residues.

Fresh mycelia (0.40 g) were homogenized in extraction solution with a mortar and pestle, diluted at a ratio of 1:50 (w/v), and shaken for 22 h at 25°C. After that, the homogenate was centrifuged at 4000 rpm for 10 min to obtain the first supernatant solution in a conical beaker. The sediment was re-suspended twice in extraction solution and shaken for 2 h at 25°C, centrifuged at 5000 rpm for 10 min, and the supernatant of the three suspending and

Table 1 Nominal and measured Cd concentrations in media and retrieval rate of nominal concentrations

Nominal concentrations (mg L^{-1})	Measured concentrations (mg L^{-1})	Retrieval rate of nominal concentration (%)
50	46.6 ± 4.4[a]	93.2
100	97.8 ± 5.7	97.8
200	189.5 ± 9.5	94.8

[a] The values are mean ± SD (n = 3)

centrifuge steps for each of the five extraction solutions were then pooled. Each of the pooled supernatant solutions was then evaporated on an electric plate at 70°C to a constant weight and digested at 145°C with an acid oxidative mixture of HNO_3:$HClO_4$ (3:1, v/v). Cd concentrations associated with the different chemical forms were determined by a flame atomic absorption spectrophotometer (AAS, model TAS990).

For Fourier transform infrared (FTIR) spectra the dry mycelia were ground with infrared-quality KBr (1:10) and pressed into disks under a vacuum using a Spectra Lab Pelletiser. The spectra (4000–400 cm^{-1}) were recorded in a Thermo Nicolet Avatar 360 FTIR spectrophotometer.

All the experiments on growth were repeated six times. Results were expressed as mean ± standard deviation (SD). Statistical analyses were performed using Duncan's new multiple range method by SPSS 11.5 software (SPSS Inc, Chicago, IL, USA).

Results and Discussion

The inhibition effects of tricyclazole on the growth of *E. pisciphila* increased with increasing tricyclazole concentration on the plate (Fig. 1 I) and in liquid media (Fig. 1 II). Colonies of the fungus were reddish brown instead of black on tricyclazole-amended media. The liquids were also reddish brown in the tricyclazole treatments. Both results indicated that tricyclazole effectively inhibited melanin synthesis in *E. pisciphila*. Furthermore, the reddish-brown level around the colony increased with increasing tricyclazole concentration in the media.

On the plate, the colony area of *E. pisciphila* increased as the culture time increased. However, the colony area of the control (0 tricyclazole) treatment was maximum and was reduced by the tricyclazole treatments. The colony areas of the tricyclazole treatments were smaller by 14.4 %–56.0 % than the control (0 tricyclazole) at 35 days. The decreased areas of the tricyclazole treatments were significant ($p < 0.05$) except for the 2.5 μg mL^{-1} tricyclazole treatment. Moreover, the colony areas of the 20 and 40 μg mL^{-1} tricyclazole treatments were significantly smaller than those of the 2.5 and 5.0 μg mL^{-1} treatments.

In the liquid media, we found no obvious differences in the mycelial biomass among the 0, 2.5, 5.0 and 7.5 μg mL^{-1} tricyclazole treatments. However, compared with the control treatment, 10, 20 and 40 μg mL^{-1} of tricyclazole caused significant decreases of 17.7, 26.1 % and 39.8 %, respectively. In addition, no obvious differences in spore numbers were seen among the 0, 2.5, 5.0, 7.5 and 10 μg mL^{-1} tricyclazole treatments, but marked decreases by 36.4 % and 60.8 % were noted for 20 and 40 μg mL^{-1}, respectively (Fig. 1 III). To summarize the above results, 2.5 μg mL^{-1} tricyclazole effectively inhibited melanin synthesis, but did not inhibit the growth of *E. pisciphila.* Thus, 2.5 μg mL^{-1} tricyclazole was nontoxic to *E. pisciphila.*

Under Cd stress, 2.5 μg mL^{-1} tricyclazole inhibited the growth (Fig. 2 I) and sporulation (Fig. 2 II) of *E. pisciphila*. Furthermore, 2.5 μg mL^{-1} tricyclazole caused obvious decreases in the mycelial biomass by 19.4 %, 40.1 % and 59.8 % and in the spore number by 33.4 %, 54.0 % and 47.4 % under 50, 100 and 200 mg mL^{-1} Cd, respectively. These results indicated that the inhibition of melanin synthesis by tricyclazole at low concentrations led to a decrease in the Cd tolerance of *E. pisciphila.*

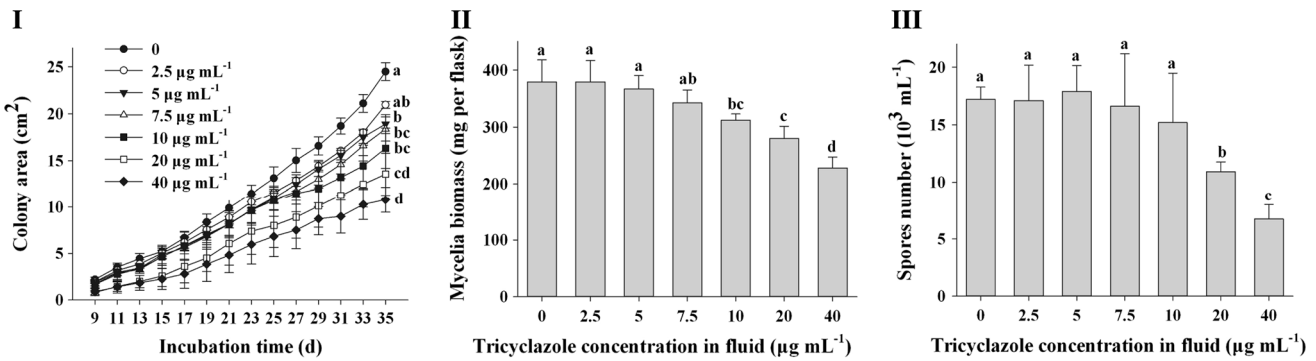

Fig. 1 Growth of *E. pisciphila* on plates (**I**), biomass (**II**) and sporulation (**III**) in liquid adding tricyclazole with different concentrations. The *bars* are the respective standard deviations (n = 5). *Different letters above the bars* indicate significant differences ($p < 0.05$), and that indicate the difference at the 35th day in (**I**)

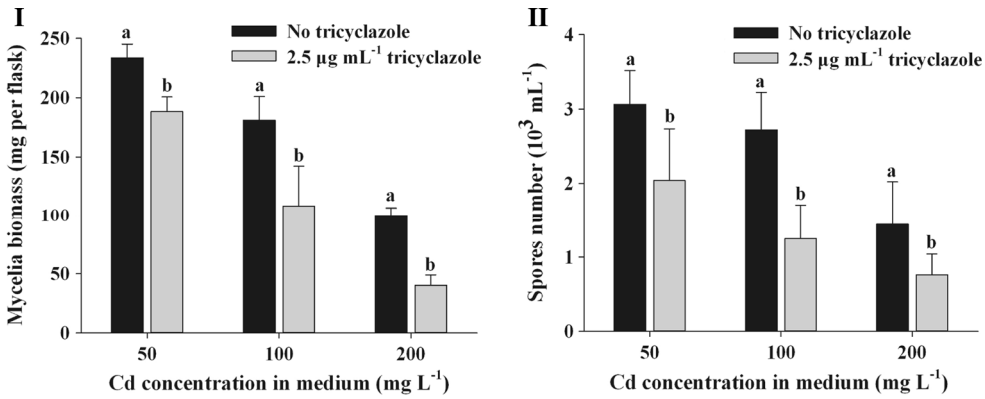

Fig. 2 Effects of tricyclazole on the mycelia biomass (**I**) and sporulation (**II**) of *E. pisciphila* under Cd stress. The *bars* are the respective standard deviations (n = 3), and *different letters above the bars* indicate significant differences ($p < 0.05$)

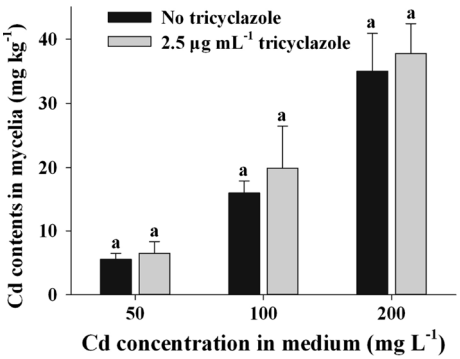

Fig. 3 Effects of tricyclazole on the Cd accumulation of *E. pisciphila*. The *bars* are the respective standard deviations (n = 3), and *different letters above the bars* indicate significant differences ($p < 0.05$)

The Cd content in the mycelia of *E. pisciphila* increased with the initial concentration of Cd in the media. Although the Cd content in the mycelia was slightly higher for 2.5 μg mL^{-1} than for the 0 tricyclazole treatment, we found no obvious differences between 0 and 2.5 μg mL^{-1} tricyclazole (Fig. 3).

The subcellular distribution of Cd in the mycelia of *E. pisciphila* was similar for 0 and 2.5 μg mL^{-1} tricyclazole. The Cd contents in the subcellular fractions of *E. pisciphila* were the cell wall ≫ the cell organelles > the soluble fraction (Table 2). No obvious differences existed between 0 and 2.5 μg mL^{-1} tricyclazole, though the Cd content in the subcellular fractions was less in the former treatment.

The distribution of the chemical forms of Cd in the mycelia of *E. pisciphila* was also similar for 0 and 2.5 μg mL^{-1} tricyclazole (Table 3). With Cd at 50 mg L^{-1} in the media, most of the Cd was extracted by 1 M NaCl (F_{NaCl}), followed by 2 % HAc (F_{HAc}). When Cd was 100 and 200 mg L^{-1} in the media, the most Cd was extracted by 2 % HAc (F_{HAc}), followed by 1 M NaCl (F_{NaCl}). Both the contents and proportion of the total Cd increased markedly following the increase of Cd in the media, while the proportion decreased by F_{NaCl} was obvious. The third largest amount was Cd in organic form extracted by deionized water (F_w) and in oxalate extracted by 0.6 M HCl (F_{HCl}). The least amount was Cd in inorganic form extracted by 80 % ethanol (F_E) and Cd in the residues (F_R). There were no obvious differences between the 0 and 2.5 μg mL^{-1} tricyclazole treatments except for F_{NaCl} and F_{HCl} at 100 mg L^{-1} Cd.

The FTIR spectra of the *E. pisciphila* mycelia were similar for the 0 and 2.5 μg mL^{-1} tricyclazole treatments (Fig. 4). The broad band at 3400–3200 cm^{-1} was due to the overlapping of the –OH and –NH stretching vibration. The peak at 2924.3 and 2923.6 cm^{-1} was indicative of –CH stretch. The band peaks at 1743.7, 1743.3, 1641.1 and

Table 2 Cadmium contents in the subcellular fraction of *E. pisciphila* mycelia (mg g^{-1})

Cd concentration (mg L^{-1})	Tricyclazole (μg mL^{-1})	Cell wall	Cell organelles	Soluble fraction	Total
50	0	$5.29^a \pm 0.31$ (93.2)	$0.31^a \pm 0.03$ (5.5)	$0.08^a \pm 0.01$ (1.4)	$5.67^a \pm 0.28$
	2.5	$5.69^a \pm 0.59$ (94.0)	$0.28^a \pm 0.04$ (4.6)	$0.08^a \pm 0.01$ (1.4)	$6.05^a \pm 0.60$
100	0	$14.32^a \pm 1.09$ (96.6)	$0.42^a \pm 0.11$ (2.8)	$0.08^a \pm 0.02$ (0.6)	$14.83^b \pm 1.83$
	2.5	$16.98^a \pm 1.36$ (96.4)	$0.55^a \pm 0.11$ (3.1)	$0.09^a \pm 0.03$ (0.5)	$17.62^a \pm 1.28$
200	0	$30.79^a \pm 2.24$ (97.2)	$0.50^a \pm 0.06$ (1.6)	$0.37^a \pm 0.03$ (1.2)	$31.67^a \pm 2.23$
	2.5	33.99 ± 1.27 (97.1)	$0.67^a \pm 0.06$ (1.9)	$0.35^a \pm 0.10$ (1.0)	$35.02^a \pm 1.24$

The content of Cd in the table is based on dry weight of fungal mycelia, values are mean ± SD (n = 3), different letters in the same row denote significant difference ($p < 0.05$)

Figures in parentheses are percent of total cadmium

Table 3 Cadmium contents of different chemical forms in *E. pisciphila* mycelia (mg g^{-1})

Cd concentration (mg L^{-1})	Tricyclazole (μg mL^{-1})	F_E	F_W	F_{NaCl}	F_{HAc}	F_{HCl}	F_R	Total
50	0	$0.014^a \pm 0.002$ (0.27)	$0.07^a \pm 0.01$ (1.2)	$3.54^a \pm 0.63$ (64.8)	$1.74^a \pm 0.27$ (31.9)	$0.09^a \pm 0.01$ (1.7)	$0.007^a \pm 0.001$ (0.12)	$5.46^a \pm 0.88$
	2.5	$0.013^a \pm 0.001$ (0.22)	$0.05^a \pm 0.01$ (0.8)	$3.65^a \pm 0.05$ (60.8)	$2.15^a \pm 0.34$ (35.9)	$0.13^a \pm 0.01$ (2.2)	$0.006^a \pm 0.001$ (0.10)	$6.00^a \pm 0.40$
100	0	$0.012^a \pm 0.001$ (0.09)	$0.08^b \pm 0.03$ (0.6)	$3.00^b \pm 0.37$ (21.0)	$10.99^a \pm 2.82$ (76.8)	$0.16^b \pm 0.01$ (1.1)	$0.051^a \pm 0.028$ (0.35)	$14.30^a \pm 2.74$
	2.5	$0.012^a \pm 0.001$ (0.07)	$0.14^a \pm 0.02$ (0.8)	$4.40^a \pm 0.13$ (25.6)	$12.20^a \pm 0.69$ (71.0)	$0.39^a \pm 0.07$ (2.3)	$0.042^a \pm 0.003$ (0.25)	$17.18^a \pm 0.76$
200	0	$0.012^a \pm 0.002$ (0.04)	$0.57^a \pm 0.06$ (1.7)	$6.14^a \pm 0.44$ (18.8)	$24.85^a \pm 3.57$ (76.2)	$0.84^a \pm 0.20$ (2.6)	$0.201^a \pm 0.039$ (0.62)	$32.61^a \pm 3.93$
	2.5	$0.009^a \pm 0.002$ (0.03)	$0.36^b \pm 0.08$ (1.0)	$7.28^a \pm 1.10$ (20.6)	$26.77^a \pm 1.40$ (75.7)	$0.79^a \pm 0.12$ (2.2)	$0.136^a \pm 0.076$ (0.38)	$35.35^a \pm 1.66$

F_E, ethanol-extractable form; F_W, water extractable form; F_{NaCl}, NaCl extractable form; F_{HAc}, acetic acid extractable form; F_{HCl}, HCl extractable form; F_R, residual form

The content of Cd in the table is based on dry weight of fungal mycelia, values are mean ± SD (n = 3), different letters in the same row denote significant difference ($p < 0.05$)

Figures in parentheses are percent of total cadmium

1640.9 cm^{-1} may be attributed to an asymmetric and symmetric stretching vibration of the C=O groups. The phosphate groups showed a characteristic peak at 1070.2–1023.2 cm^{-1} representing the presence of phosphate groups. Thus, there were hydroxyl, amine, carboxyl and phosphate groups in the mycelia of *E. pisciphila*. These groups were potential binding groups to Cd. 2.5 μg mL^{-1} tricyclazole did not obviously change the functional groups in the mycelia of *E. pisciphila*.

The reddish-brown instead of black colonies of *E. pisciphila* on tricyclazole-amended media indicated that tricyclazole effectively inhibited DHN melanin synthesis. Tricyclazole blocked the DHN melanin pathway by inhibiting the reduction of 1,3,6,8-tetrahydroxynaphthalene (THN) to scytalone and 1,3,8-THN to vermelone, and caused the endogenous accumulation of the melanin intermediates flaviolin, 2-hydroxyjuglone (HJ) and 3-HJ in media. These intermediates were secreted into the media and produced a reddish-brown color (Geis et al. 1984; Romero-Martinez et al. 2000).

Generally, the minimum concentration of tricyclazole required to inhibit DHN melanin synthesis varies from <0.1 to 10 μg mL^{-1} for different species (Bell and Wheeler 1986). Tricyclazole at a low concentration was considered nontoxic since it only inhibited melanization but not mycelial growth or conidial germination (Elliott 1995; Tokousbalides and Sisler 1978). Tricyclazole reduced fungal growth as its concentrations increased (Fernando and Bean 1986), but it caused a marked toxicity only when its concentration was very high, such as a 50 % inhibition (ED_{50}) for tricyclazole on the growth of *Magnaporthe oryzae* of 100.41 mg L^{-1} (Kunova et al. 2013). In this study, 2.5 μg mL^{-1} tricyclazole had no apparent effect on growth and sporulation, except for the inhibition of melanization. So it can be considered be nontoxic to *E. pisciphila*.

Melanin is a structural component for the integrity of the cell wall of fungi (Pihet et al. 2009) or a cross-linker of cell wall components (Eisenman et al. 2005; Zhong et al. 2008). Melanin is deposited in the inner or outer cell wall layer in large granules and forms a firm, thick, shield-like layer on the cell wall to protect the fungi against environmental stresses (Kogej et al. 2006; Youngchim et al. 2004). Additionally, melanin has high activity in binding metal ions and protects the fungi against heavy metals (Buszman et al. 2006). The addition of tricyclazole inhibited melanin synthesis, caused the melanin layer to be absent and the cell wall to be thinner (Caesar-Tonthat et al. 1995; Pihet et al. 2009), or prevented melanin-cross linking within cell walls (Franzen et al. 2006). The lack of melanin decreased cell wall strength and increased cell wall porosity or permeability (Henson et al. 1999). This left the fungus more susceptible to the toxicity of heavy metals and

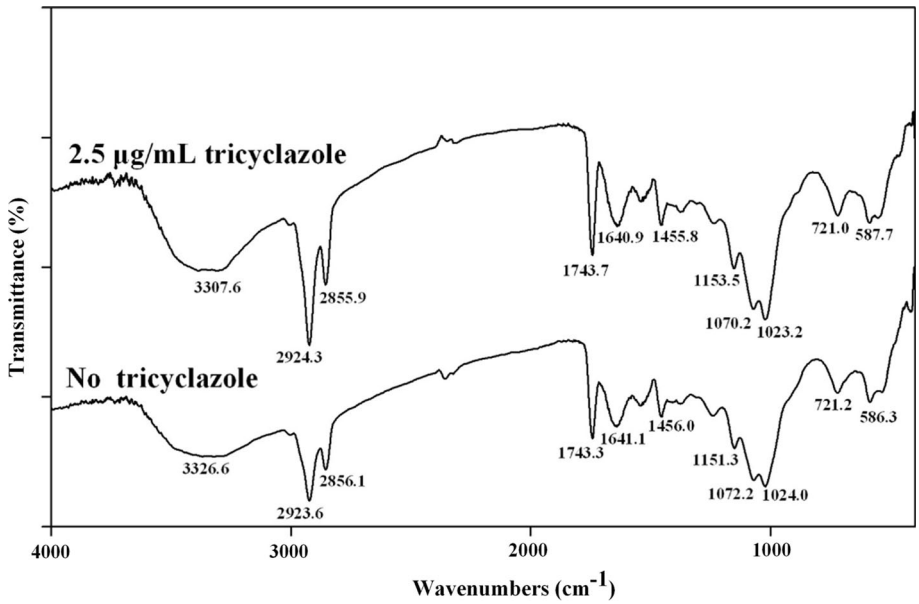

Fig. 4 FTIR spectra of *E. pisciphila* mycelia under 200 mg L^{-1} Cd stress

resulted in a decrease in the tolerance of fungi to heavy metals. Hence, inhibition of DHN melanin synthesis by 2.5 μg mL^{-1} tricyclazole decreased Cd tolerance in *E. pisciphila*.

The primary components of the mycelia are responsible for the accumulation of heavy metals in fungi (Gaur et al. 2014). Fungal mycelia are mostly comprised of glycoproteins and polysaccharides, which mainly include glucan, chitin and chitosan (Latge 2007). Tricyclazole had no apparent effect on the synthesis of the main components of fungal mycelia except for inhibiting the synthesis of DHN melanin. This was also confirmed by the FTIR spectra of *E. pisciphila* mycelia, which did not exhibit obvious changes with an addition of tricyclazole. Furthermore, melanin is only a minor part of fungal mycelia; for example, the melanin content was 9.05 to 12.10 mg g^{-1} dry biomass in *E. pisciphila* (Zhan et al. 2011). So inhibition of DHN melanin synthesis by tricyclazole did not alter the accumulation characteristics of Cd in the mycelia of *E. pisciphila*.

It is noteworthy that tricyclazole is a common fungicide used to control crop diseases caused by melanized fungi, such as rice blast fungus, *Magnaporthe grisea* (Zhang et al. 2009), and maize leaf spot fungus, *Setosphaeria turcica* (Guo et al. 2012). Tricyclazole is mostly sprayed on the crop leaves and is easily spread from the leaves into the soils and residues in farmlands because of its water solubility (Jeong et al. 2012; Padovani et al. 2006; Phong et al. 2009; Tsochatzis et al. 2013). These residual tricyclazoles inevitably affect the melanized fungi in farmlands. Many studies have found that the inhibition of melanin synthesis by tricyclazole resulted in certain changes in fungal growth and physiological activities, such as the inhibition of some secondary metabolites (Lee et al. 2003; Tokousbalides and Sisler 1978), aflatoxin release reduction (Fernando and Bean 1986), and a decrease in infection efficiency (Kunova et al. 2013). As a result, tricyclazole has a marked impact on the tolerance of melanized fungi against environmental stresses (Cunha et al. 2005). Thus, it is necessary to carry out further studies on the potential effects of tricyclazole on non-target fungi in soils and to evaluate its risk assessment in environment.

Acknowledgments This work was financially supported by the National Natural Science Foundation of China (Nos. 41101486, 41461093, 31460114 and U1202236), and Key Research Project of Education Bureau of Yunnan Province, China (No. 09Z0037).

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