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Phenolic acids in the plow layer soil of strawberry fields and their effects on the occurrence of strawberry anthracnose

Geilin Tian · Yanmeng Bi · Zhenjun Sun · Lusheng Zhang

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Abstract Phenolic allelochemicals have been found in both natural and managed ecosystems, where they cause numerous ecological and economic problems. Whether these problems can be mediated by some other specific phenolic acid components is unknown. In this study, we identified phenolic acids and their concentrations in plow layer soil, rhizosphere soil and decomposing strawberry (*Fragaria ananassa* Duch.'Benihoppe'.) plants susceptible to strawberry anthracnose crown rot. We also assessed the effects of exogenously added phenolic acids at varying concentrations on *Colletotrichum gloeosporioides*, the pathogen causing strawberry anthracnose crown rot, conidial germination and colony growth. Finally, we verified the occurrence of strawberry anthracnose crown rot and the changes in root structure in response to phenolic acids. Ten phenolic acids were identified in soil samples. The concentrations of *p*-coumaric acid (PA) and ferulic acid (FA) were higher than other phenolic acids. Relatively high concentrations of PA and FA could increase the occurrence of strawberry seedling anthracnose crown rot. However, when the concentrations of PA and FA were higher than respectively certain critical concentration, they could reduce the degree of the disease. Meanwhile, high concentration of FA seriously inhibited the growth of root. The *trans*-cinnamic acid (TA) content could be regulated to control the occurrence of strawberry anthracnose crown rot without affecting root growth. Overall, diverse phenolic acids in plow soil had different influence on strawberry anthracnose crown rot. The effects of phenolic acids were concentration-dependent and *C. gloeosporioides* was more sensitive to phenolic acids concentration than root.

Keywords Phenolic acid · Plant–microbe interaction · Strawberry anthracnose crown rot · Root exudates · Allelopathy

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Introduction

Allelochemicals are released by plant leaching, root exudation, volatilisation and residue decomposition, and are typically detrimental to crop performance (Weir et al. 2004; Jilani et al. 2008). Plant–microbe interaction research has revealed that plants are able to shape their rhizosphere microbiome (Berendsen et al. 2012) and can modify the soil microbial community (Kong et al. 2008; Lorenzo et al. 2013).

Low-molecular-weight organic compounds in root exudates or decomposition residues play an important role in plant–microorganism interactions by influencing the structure and function of soil microbial communities (Shi et al. 2011). These compounds have been isolated and identified mainly as organic acids, especially phenolic acids such as *trans*-cinnamic acid (TA), vanillic, *p*-coumaric acid (PA), ferulic acid (FA) and salicylic acid et al., (Yu and Matsui 1994; He et al. 2008; Hao et al. 2010; Huang et al. 2010; Zhou and Wu 2013). When the microbial community in soils becomes less diverse, beneficial species decrease or disappear, while populations of harmful species and pathogens increase (Chen et al. 2011a, b). Exogenously applied *p*-hydroxybenzoic acid plays a significant role in the chemical interaction between cucumber (*Cucumis sativus* L.) and soil microorganisms, and could account for the changes in soil microbial communities in continuously mono-cropped cucumbers (Zhou et al. 2012a). Moreover, exogenously applied salicylic acid (Wu et al. 2008a, b), sinapic acid (Wu et al. 2009a), FA (Wu et al. 2010a, b; Zhou and Wu 2012a, b), PA (Zhou and Wu 2012a, b), tannic acid (Wu et al. 2010a, b), gallic acid (Wu et al. 2009b) and TA (Ye et al. 2004) have been shown to influence the rhizosphere soil microbial communities and the growth of *Fusarium oxysporum* f.sp. Application of beneficial microorganisms that biodegrade phenolic acid allelochemicals is a highly efficient way to resolve the problems associated with continuous cropping systems (Chen et al. 2011a, b; Huang et al. 2013). But previous studies also had provided in planta evidence that chlorogenic acid may play a role in maize resistance to Gibberella ear rot and trichothecene accumulation (Atanasova-Penichon et al. 2012) and natural phenolic acids from wheat bran could inhibit *Fusarium culmorum* trichothecene biosynthesis in vitro by repressing *Tri* gene expression (Boutigny et al. 2010).

Strawberry (*Fragaria ananassa* Duch.) is a crop of economic importance (Cao and Wang 2007), and strawberry anthracnose is one of the most serious diseases affecting its survival (Salazar et al. 2007; Widiastuti et al. 2013). *C. gloeosporioides* causes strawberry anthracnose crown rot, a destructive disease typically found in strawberry nurseries (MacKenzie et al. 2006; Garrido et al. 2007); it can survive in soil (Freeman et al. 2002) and favours a continuous cropping system (Larry 2008). Phenolic compounds have been implicated as autotoxins of strawberry under mono-cropping management systems (Kitazawa et al. 2005; Cao and Wang 2007; Asaduzzaman et al. 2012). The response of three root rot fungi to strawberry phenolics and the relationship of phenolics to disease resistance were different (Nemec 1976) and the combination of *p*-hydroxybenzoic acid and *F. oxysporum* has a negative effect on leaf photosynthesis (Zhao et al. 2009).

More and more cash crops are suffering from different kinds of devastating soil-borne diseases that limit crop production, especially in continuously monocropping systems (Hao et al. 2010). Strawberry anthracnose crown rot is prevalent in strawberry nurseries, but the relationship between phenolic acids in the plow soil layer and the occurrence of anthracnose crown rot has not been extensively studied. Application of beneficial microorganisms that biodegrade phenolic acid allelochemicals is a highly efficient way to control soil borne fungi disease (Chen et al. 2011a, b). The increase of phenolic acids in plant organs could prevent many fungal disease (Atanasova-Penichon et al. 2012), but control soil borne fungi disease by exogenous adding phenolic acids has not been studied. Plant can produce a large amount of phenolic components by root exudation and residue decomposition. The research of reduce soil borne fungal disease by exogenous adding phenolic acids is the first step in developing a strategy by allelopathy to reduce the soil borne disease. We put forward a hypothesis that soil borne disease can be mediated by adding special phenolic acids and we anticipate that the results from our research will contribute to the control of strawberry anthracnose crown rot by regulating phenolic acids. The objectives of this study were to identify the phenolic compounds in the plow layer soil in mono-cropping management systems and to determine the inhibitory or stimulation effects of these compounds on *C. gloeosporioides*, as well as observe the effects of adding phenolic acids to the plow layer of soil at varying concentrations on the occurrence of strawberry anthracnose and the impact on root growth.

Materials and methods

Collection of soil and decomposition of stalk samples

An experiment was conducted in plastic pots (15 cm diameter × 15 cm height) in a glasshouse located at the experimental station of the China Agricultural University Science Centre, Beijing, China, in September 2012. The PAR in glasshouse at noon was controlled below

1000 μmol m^{-2} s^{-1}. The temperature maintained between 20 and 25 °C. Air relative humidity maintained 70–80 % and photoperiod were about 12 h light/12 h dark. In this study, the strawberry cultivar 'Benihoppe' which was purchased from Institute of Forestry and Pomology Beijing Academy of Agricultural and Forestry Sciences is susceptible to strawberry anthracnose crown rot and widely cultivated all over the world. Each pot contained one strawberry plant, and raw soil was taken from the Shang Zhuang test station of the China Agricultural University. Cultivated soil was mixed with raw soil, roseite, glassy carbon and river sand at a ratio of 5:2:2:1. The cultivated soil contained the following: organic matter, 2.07 %; available N (nitrate and ammonium), 59.02 mg kg^{-1}; Olsen P, 41.36 mg kg^{-1}; available K, 79.15 mg kg^{-1}; pH (1:2.5, w:v), 7.78. The strawberry plants were normal irrigation management and soil moisture maintained 18 %. 60d after transplanting, the strawberry roots were carefully separated from the soil by inverting the pots. Only the soil adhering to the roots was considered as rhizosphere soil (Nazih et al. 2001), which was collected by shaking off soil from the roots (Xu et al. 2009) and sieving (2-mm mesh) to remove the root tissues for subsequent analysis. The samples of rhizosphere soil were respectively collected by shaking off from the roots of six different potted seedlings of strawberry and respectively identified phenolic acids and their concentrations.

The strawberry cultivar 'Benihoppe' was used and the plow layer soil samples were collected during July 2013 from the plow layer soil of nursery fields in Chang-Ping County, Beijing, China. The samples of plow layer soil were collected by drilling with a soil auger at six different points accordance with the principle "S" and respectively identified phenolic acids and their concentrations. Strawberry stalks and leaves were dried and pulverised, followed by mixing with deionised water at a 3:1 ratio of water to dried sample. A small amount of soil was added to decompose in a glass culture bottle at room temperature (20–30 °C) for 30 d. The decomposing test had six biological replicates with six glass culture bottles in the same batch. The decomposed stalks and leaves were analysed for phenolic acid composition.

Phenolic acid extraction and determination

Phenolic acids were extracted from soil, decomposed stalks and leaves with methods previously described by Dalton et al. (1987). In total, 25 g fresh soil or 0.5 g decomposed stalk was added to 25 ml or 1 ml 1 M NaOH, respectively, and stored for 24 h. The suspension was centrifuged at 8000 × *g* for 10 min to separate the liquid supernatant. The pH of the supernatant was adjusted to 2.5 with 12 M HCl and stored for 2 h. The suspension was centrifuged at 8000 × *g* for 10 min. The supernatant was stored at 4 °C and prepared for analysis.

The solution of soil extracts was filtered through a 0.22-μm microporous membrane filter for subsequent high-performance liquid chromatography (HPLC) analysis (model 1260, Agilent, Waldbronn, Germany). Phenolic acid separation was performed by gradient elution high-performance liquid chromatography (GEHPLC) with an HPLC quaternary pump model G1311A, a UV detector model G1314A and a chromatographic column: XDB-C18 (4.6 mm×250 mm). The detector was set to 280 nm and the flow rate to 1 ml/min. The injection volume was 20 μL and the column temperature was maintained at 25 °C. Methanol (A) and acetic acid solution (pH=2.80) (B) were used as mobile phases with a gradient elution B: 70 % (0 min)→50 % (15 min)→30 % (16 min)→0 (30 min)→end (30 min). Phenolic compounds were identified and quantified by comparing retention times and areas with pure standards. The concentrations of each compound in the soil samples were determined based on peak areas using external standards and expressed as milligrams per gram of dry soil. Standard phenolic acids used for HPLC analysis were gallic acid, PA, syringic acid, *p*-hydroxybenzoic acid, FA, TA, chlorogenic acid, caffeic acid, protocatechuic acid, vanillin and benzoic acid. All purchased chemicals were of high purity and the solvents used were HPLC spectral grade. Eleven phenolic acid standards were purchased from Sigma-Aldrich (St. Louis, MO).

Fungal isolates and interaction between phenolic acid and *C. gloeosporioides* in vitro

The strawberry anthracnose pathogen *C. gloeosporioides* was isolated from a plant with strawberry crown rot symptoms in commercially cultivated glasshouse fields by Professor Zhang Guozhen at the College of Agriculture and Biotechnology, China Agricultural University. Isolation was based on morphological criteria and molecular methods established in 2013. Inocula were prepared from 6 to 8 day old cultures grown at 24 °C on potato dextrose agar. Conidial suspensions used for

inoculation were prepared in sterile deionised water, filtered through four layers of cheesecloth and diluted to 1×10^6 conidia/ml (MacKenzie et al. 2006). Measurement of *C. gloeosporioides* colony growth and assessment of its conidial germination were according to methods previously described by Wu et al. (2008b).

Effect of phenolic acids on the occurrence of strawberry anthracnose by *C. gloeosporioides*

An experiment was conducted using plastic pots (10 cm diameter×10 cm height) in a glasshouse located at the experimental station of the China Agricultural University Science Centre, Beijing, in September 2014. Field soil samples were taken from the Shang Zhuang test station of the China Agricultural University. The PAR in glasshouse at noon was controlled below 1000 μmol m^{-2} s^{-1}. The temperature maintained between 20 and 30 °C. Air relative humidity maintained 70–80 % and photoperiod were about 12 h light/12 h dark. Cultivated soil was mixed with field soil, roseite, organic fertilizer and glassy carbon at a ratio of 5:2:1:2. The basic properties of the mixed medium were organic matter, 3.12 %; available N (nitrate and ammonium), 82.35 mg kg^{-1}; Olsen P, 59.46 mg kg^{-1}; available K, 129.25 mg kg^{-1}; pH (1:2.5, w:v), 7.68. Seedlings with 3–4 leaves were selected and assigned to the 12 treatments described in Table 1. Each treatment had 12 replicates. Three strawberry plants were in a given pot and the weight of dry soil was 200 g. The strawberry plants were normal irrigation management and soil moisture maintained 20 %.

Our previous study found that the concentrations of PA or FA in soil samples ranged from 5.53±0.38 to 81.64±12.02 μg g^{-1} dry soil and the concentration of total phenolic in strawberry field ranged from 139.06± 6.38 to 250.72±19.02 μg g^{-1} dry soil (data not shown). Considering synergy and antagonism between different phenolic acids, we choose the corresponding concentration to test. Due to microbial utilisation of the compounds under favourable environmental conditions, previous studies have shown that phenolic compounds could be depleted rapidly after being added to the soil (Blum and Shafer 1988). In this study, different phenolic acids at varying concentrations were added to the soil every 2 d to maintain the desired levels. Phenolic acids were dissolved in methanol and diluted with distilled water. The concentration of each phenolic acid was established at 200 mg l^{-1}, and the solution pH was adjusted to 7.0 with 0.1 M NaOH solution. Soil treated with distilled water was used as the control. Conidial suspensions used for the inoculation solution contained 1.0×10^6 *C. gloeosporioides* spores per milliliter and the way of inoculation was irrigating strawberry plants to topsoil with conidial suspensions before phenolic acid treatments.

Crown rot symptoms started after 20 d of inoculation in topsoil, and symptoms on the petiole base and longitudinal sections of the main stem were evaluated after 30 d. The degree of petiole disease occurrence was rated on

Table 1 Twelve different treatment concentrations of phenolic acids on the occurrence of strawberry anthracnose crown rot by *C. gloeosporioides* in pot experiment

Treatment	Concentrations of desired phenolic acid	Phenolic acid and water in first day	Phenolic acid and water every 2 days	Spore suspension in first day
low concentration FA (LF)	25 μg/g (dry soil)	25.0 ml+75.0 ml	12.5 ml+87.5 ml	0 ml
low concentration FA + *C. gloeosporioides* (LFC)	25 μg/g (dry soil)	25.0 ml+75.0 ml	12.5 ml+87.5 ml	80 ml
low concentration PA (LP)	25 μg/g (dry soil)	25.0 ml+75.0 ml	12.5 ml+87.5 ml	0 ml
low concentration PA + *C. gloeosporioides* (LPC)	25 μg/g (dry soil)	25.0 ml+75.0 ml	12.5 ml+87.5 ml	80 ml
high concentration FA (HF)	100 μg/g (dry soil)	100.0 ml+0 ml	50.0 ml+50.0 ml	0 ml
high concentration FA + *C. gloeosporioides* (HFC)	100 μg/g (dry soil)	100.0 ml+0 ml	50.0 ml+50.0 ml	80 ml
high concentration PA (HP)	100 μg/g (dry soil)	100.0 ml+0 ml	12.5 ml+87.5 ml	0 ml
high concentration PA + *C. gloeosporioides* (HPC)	100 μg/g (dry soil)	100.0 ml+0 ml	12.5 ml+87.5 ml	80 ml
low concentration TA (LT)	25 μg/g (dry soil)	25.0 ml+75.0 ml	50,0 ml+50.0 ml	0 ml
low concentration TA + *C. gloeosporioides* (LTC)	25 μg/g (dry soil)	15.0 ml+75.0 ml	50.0 ml+50.0 ml	80 ml
CK	natural level	0 ml+100.0 ml	0 ml+100.0 ml	0 ml
CK+ *C. gloeosporioides* (CKC)	natural level	0 ml+100.0 ml	0 ml+100.0 ml	80 ml

a scale of 0–4 (Delp and Milholland 1980) as follows: 0 (whole plant was healthy); 1 (<3.0 mm of dot length); 2 (1–20 mm of dot length); 3 (>20 mm of dot length); 4 (whole plant dead). The degree of crown disease occurrence was rated on a scale of 0–4 as follows: 0 (whole plant was healthy); 1 (<¼ of the ratio of decay area and longitudinal section); 2 (¼–½ of the ratio of decay area and longitudinal section); 3 (½–¾ of the ratio of decay area and longitudinal section); 4 (>¾ of the ratio of decay area and longitudinal section and the whole plant died). The disease index (DI) and disease rate (DR) were calculated as follows:

$$DI = \left[\sum (X_i \times S_i)/(S_{max} \times N)\right] \times 100$$
$$DR = n/N \times 100$$

where X_i is the number of strawberry plants with the corresponding disease index, S_i is the disease index, S_{max} is the highest disease index in the diseased strawberry plants, N is the total number of strawberry plants and n is the number of susceptible strawberry plants.

Root structure analysis

The roots that slowly thawed were scanned with a double scan scanner (Expression 1600 Pro, model EU-35, Epson, Tokyo, Japan). Scanning images were obtained using a WinRHIZO image analysis system (WinRHIZO Pro2004, version 5.0, Regent Instruments, Quebec, ON, Canada) and used to measure the total root length (cm), root surface area (cm^2), average root diameter (mm), root volume (cm^3), root tips and root forks.

Statistical analysis

Data were analysed using Excel™ (Microsoft, Redmond, WA) as means over replicates (mean±SE) for each treatment. Duncan's multiple range test was applied when one-way ANOVA revealed significant differences ($P<0.05$). All statistical analyses were performed with SPSS Base Version 18.0 statistical software (SPSS Inc., Chicago, IL).

Results

Identification of phenolic acids in the plow layer soil, rhizosphere soil and decomposition of stalk and leaf of strawberry plants

The phenolic compounds obtained from the rhizosphere soil, plow layer soil and decomposed strawberry stalks and leaves were analysed using HPLC. Phenolic acids were identified in the chromatograms shown in Fig. 1. Ten phenolic acid compounds were detected from rhizosphere soils samples (in the order of their appearance in the eluant): (1) gallic acid, (2) protocatechuic acid, (3) chlorogenic acid, (4) *p*-hydroxybenzoic acid, (5) caffeic acid, (6) syringic acid, (7) vanillin, (8) PA, (9) FA and (10) TA (Fig. 1b). Protocatechuic acid was not detected from the plow layer and chlorogenic acid was not detected in decomposed stalks and leaves (Table 2). The content of PA was higher than other phenolic acids, with concentrations of 27.09±3.23 μg g^{-1} in the plow layer soil and 81.64±12.02 μg g^{-1} in decomposed stalks and leaves. The content of FA was second highest, with 5.53±0.38 μg g^{-1} in the plow layer soil and 39.53±1.90 μg g^{-1} in decomposed stalks and leaves. The content of FA was higher than other phenolic acids and the concentrations were 32.19±2.23 μg g^{-1} but the concentration of PA was 16.18±1.14 μg g^{-1} in the rhizosphere soil at the seedling stage. Contents of the remaining phenolic acids, including gallic acid, protocatechuic acid, chlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid, syringic acid, vanillin and TA, were very low.

Effects of phenolic compounds on the growth of *C. gloeosporioides*

The phenolic compounds showed various degrees of dose-dependent inhibition or promotion in colony growth of *C. gloeosporioides* (Table 3). Colony growth on plates was stimulated at a low PA concentration (0–50 mg l^{-1}) and increased from 7.09±0.04 cm (0 mg l^{-1}) to 7.55±0.12 cm (50 mg l^{-1}). Colony growth was suppressed at high concentrations of PA from 7.55±0.12 cm (50 mg l^{-1}) to 0±0 cm (800 mg l^{-1}). Increasing the concentration of FA from 0 mg l^{-1} to 50 mg l^{-1} had no effect on colony growth of *C. gloeosporioides*, but at high FA concentrations, colony growth decreased from 7.12±0.09 cm (50 mg l^{-1}) to 3.54±0.20 cm (800 mg l^{-1}). Most of the phenolic acid compounds

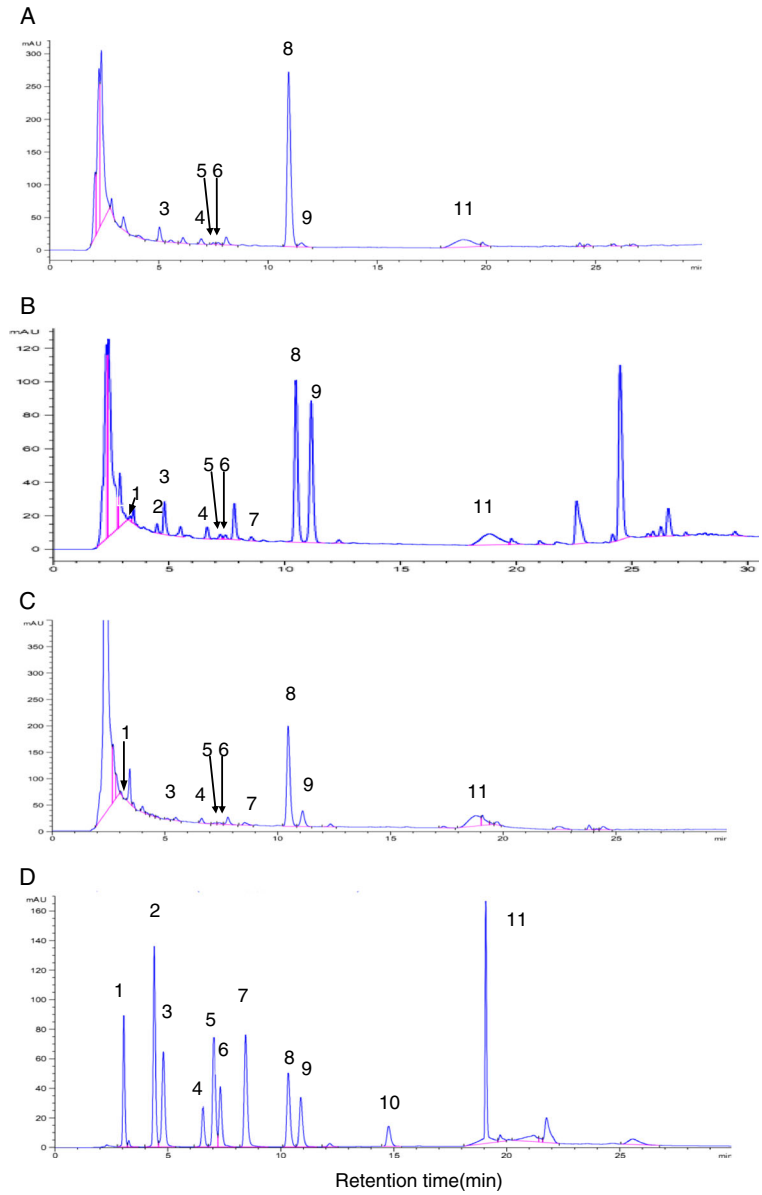

Fig. 1 Representative HPLC chromatogram from the extraction of the plow layer soil (**a**), rhizosphere soil of seedling stage (**b**), decomposition of stalk and leaf (**c**) and Standards (**d**). Compounds detected from samples (in the order of their appearance in the eluant): (1) gallic acid, (2) protocatechuic acid, (3) chlorogenic acid, (4) P-hydroxybenzoic acid, (5) caffeic acid, (6) syringic acid, (7) Vanillin, (8) PA, (9) FA, (10) benzoic acid and (11) TA

(PA, FA, chlorogenic acid, gallic acid, chlorogenic acid, protocatechuic acid, syringic acid, vanillin and caffeic acid) showed a variety of promoting effect or no effect on colony growth at low concentrations and inhibited growth at high concentrations. The concentrations of the transition from the promotion to inhibition of colony growth varied among the phenolic acids. TA inhibited colony growth at a concentration of 50 mg l^{-1}, and it had lower concentration range changes going from promoting to inhibiting the growth of *C. gloeosporioides* colonies.

Effects of different phenolic acids at varying concentrations on conidial germination of *C. gloeosporioides*

The effects of different concentrations of exogenous PA and FA on conidial germination of *C. gloeosporioides* are shown in Fig. 2. With an increase in the FA concentration of less than 50 mg l^{-1}, conidial germination of *C. gloeosporioides* was enhanced. Conidial germination of *C. gloeosporioides* in a treatment of 50 mg l^{-1} FA was 34.25±1.71, while the control (0 mg l^{-1} FA) was 27.50±

Table 2 Concentration of phenolic acids in rhizosphere soil, plow layer soil and decomposition of stalk and leaf of strawberry plants (μg g^{-1}dry soil)

Phenolic acids	Rhizosphere soil	Plow layer soil	Decomposition of stalk and leaf
gallic acid	1.87±0.22	2.37±0.15	2.40±0.20
protocatechuic acid	1.16±0.07	–	0.87±0.09
chlorogenic acid	10.23±1.85	6.29±0.76	–
p-hydroxy benzoic acid	1.48±0.11	0.71±0.08	1.52±0.13
caffeic acid	0.61±0.02	0.59±0.04	0.76±0.09
syringic acid	1.04±0.08	0.98±0.08	1.13±0.07
Vanillin	3.14±0.12	4.25±0.33	5.57±0.15
PA	16.18±1.14	27.09±3.23	81.64±12.02
FA	32.19±2.23	5.53±0.38	39.53±1.90
TA	3.06±0.28	3.93±0.36	4.46±0.81

Note."–" indicate failed to detect the content of corresponding phenolic acid

1.91. When the concentration of FA was greater than 50 mg l^{-1}, increased inhibition of conidial germination occurred. Conidial germination in a treatment of 200 mg l^{-1} FA was 0±0.00 (Fig. 4). Conidial germination of *C. gloeosporioides* was inhibited at a low PA concentration, ranging from 25 mg l^{-1} (21.25±0.96) to 400 mg l^{-1} (0.00±0.00). Conidial germination of the control (0 mg l^{-1} PA) was 27.50±1.91.

The effects of exogenous protocatechuic acid, chlorogenic acid, caffeic acid, gallic acid and *p*-hydroxybenzoic acid on conidial germination of *C. gloeosporioides* are shown in Fig. 3. With an increase in chlorogenic acid concentration of less than 100 mg l^{-1}, conidial germination was promoted. When the concentration of chlorogenic acid was greater than 100 mg l^{-1}, a reduced inhibition of conidial germination was observed. Caffeic acid and chlorogenic acid had similar effects. Protocatechuic acid did not significantly affect conidial germination. Inhibition of conidial germination of *C. gloeosporioides* from 68.75±2.98 (0 mg l^{-1}) to 38.75±5.06 or 28.75±6.29 (400 mg l^{-1}) by *p*-hydroxybenzoic acid and gallic acid, respectively, were observed with an increase in their concentrations.

Table 3 Effects of exogenous ten phenolic compounds on the colony growth of *C. gloeosporioides* (after 6d)

Phenolic acid	Concentration (mg l^{-1})					
	0	50	100	200	400	800
PA	7.09±0.04b	7.55±0.12a	7.17±0.08b	6.64±0.20c	4.40±0.03 d	0±0.00e
FA	7.09±0.04a	7.12±0.09a	6.34±0.16b	5.56±0.14c	4.24±0.08	3.54±0.20e
Gallic acid	7.37±0.13ab	7.49±0.09a	7.40±0.12ab	7.39±0.02ab	7.29±0.25b	6.84±0.08c
Chlorogenic acid	7.37±0.13a	7.38±0.03a	7.40±0.08a	7.43±0.05a	7.45±0.03a	5.26±0.06b
P-hydroxybenzoicacid	7.37±0.13a	7.45±0.11a	7.42±0.06a	7.41±0.05a	7.38±0.03a	7.32±0.02a
Caffeic acid	7.37±0.13a	7.34±0.09a	7.38±0.14a	7.39±0.12a	6.85±0.05b	6.51±0.22c
Protocatech-uic acid	7.37±0.13a	7.44±0.07a	7.32±0.09a	7.31±0.06a	7.29±0.13a	6.98±0.12b
TA	7.25±0.08a	6.92±0.21b	6.62±0.16c	6.41±0.07d	5.14±0.11e	0.00±0.00f
Syringic acid	7.25±0.08a	7.29±0.11a	7.22±0.12a	7.09±0.06b	7.08±0.06b	6.73±0.10c
Vanillin	7.25±0.08a	7.27±0.04a	7.26±0.06a	7.18±0.10ab	7.06±0.07b	6.84±0.09c

The data represent mean±SD (n=5) of colonydiameter (cm). Values in the same row followed by the same level case letter are not statistically different at P=0.05 by Duncan's test. The reason of the colony growth was different at the 0 concentration of phenolic acid was different batches of test

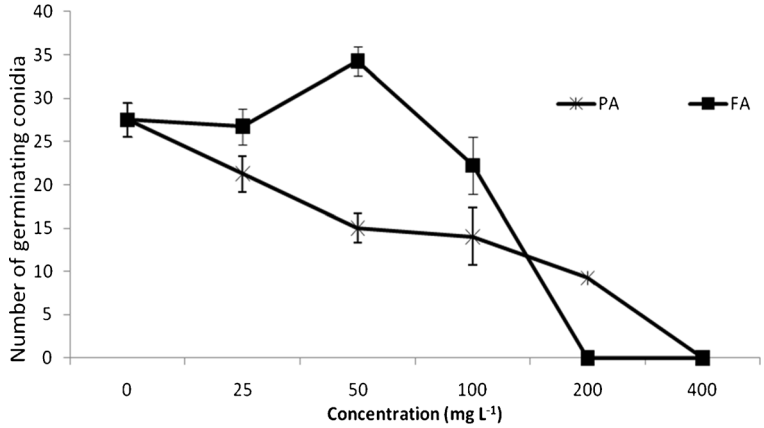

Fig. 2 Effects of different concentration of PA acid and FA on conidial germination of *C. gloeosporioides* (after 48 h). *Bars* indicate 2 times standard error

The effects of exogenous syringic acid, vanillin acid, and TA on conidial germination of *C. gloeosporioides* are shown in Fig. 4. Conidial germination was not significantly affected by syringic acid and vanillin acid at concentrations from 25 to 400 mg l^{-1}, while TA inhibited conidial germination significantly at varying concentrations. Conidial germination for TA treatments of 25 and 50 mg l^{-1} was 10.75±1.70 and 0, respectively. Germination for the control was 22.75±2.51.

Effect of phenolic acids on the occurrence of strawberry anthracnose by *C. gloeosporioides* in a pot experiment

The effects of the continuous delivery of different phenolic acids, and at varying concentrations, on the occurrence of strawberry anthracnose crown rot in a pot experiment are shown in Table 4. Crown rot symptoms which refer to a small amount of petioles base appear dot symptoms started after 20 d of inoculation in topsoil. A small number of leaves gradually wilted (Fig. 5a) and begun to appear plant whole death between 20 to 30 d and symptoms on the petiole base and longitudinal sections of the main stem (Fig. 5b) were evaluated after 30 d. The treatment of a LFC compared with CKC, significantly promoted the incidence of anthracnose symptoms. The DI, DR, average individual plant crown rot scale and total crown rot rate were 34.88±15.89, 40.38 %, 1.25±0.70 and 66.67 %, respectively. For the CKC, DI, DR, average individual plant crown rot scale and total crown rot rate were only 21.32±11.02, 28.12 %, 0.53±0.34 and 41.76 %, respectively. The effects of a LPC on the occurrence of strawberry anthracnose were similar to the treatment of a LFC. No significant differences were observed between the two treatments. No significant differences between the effects of a HPC and HFC were detected compared with the CKC. The treatment of a LTC, compared with the CKC, significantly suppressed the incidence of anthracnose.

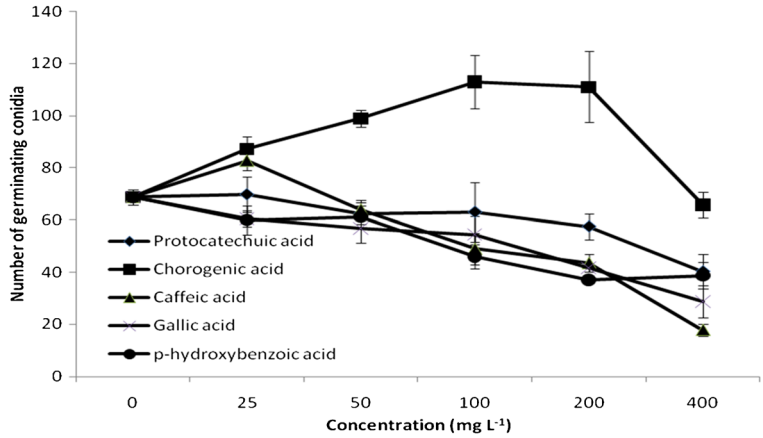

Fig. 3 Effects of different concentration of protocatechuic acid, chlorogenic acid, caffeic acid, gallic acid and p-hydroxybenzoic acid on conidial germination of *C. gloeosporioides* (after 48 h). *Bars* indicate 2 times standard error

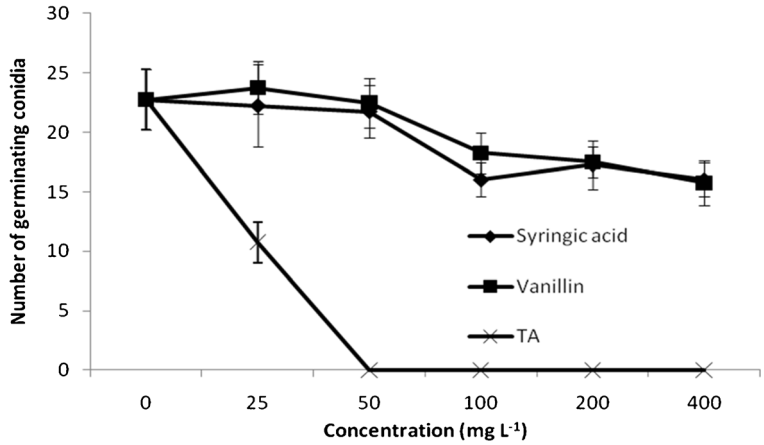

Fig. 4 Effects of exogenous syringic acid, vanillin, and TA on conidial germination of *C. gloeosporioides* (after 48 h). *Bars* indicate 2 times standard error

Effect of phenolic acids on root of strawberry plants

The effects of different phenolic acids at varying concentrations on the total root length, root surface area, average root diameter, root tips and root volume are shown in Table 5. At a low concentration, no significant differences were detected for the total root length, root surface area, average root diameter, root tips and root volume. A high concentration of FA significantly reduced the total root length, root surface area, root tips and root volume compared with the other phenolic acids at a low concentration and PA at a high concentration.

The effects of phenolic acids at varying concentrations on above-ground fresh weight varied. The above-ground fresh weight of LT was significantly higher than for LF, HP, HF and CK. The treatment of HF significantly limited root growth (Fig. 6a), but the effects on above-ground fresh weight were not significantly different for low concentrations of FA, PA, high concentration of PA and the control. Total fresh weight and above-ground fresh weight are shown in Fig. 6b. No significant differences were observed between the phenolic acid treatments at a low concentration, but the root fresh weight (1.31±0.19 g) for the high FA treatment was significantly lower than for low concentrations of FA (2.66±0.47 g), PA (2.24±0.34 g), TA (2.63±0.33 g), high concentrations of PA (2.18±0.35 g) and the control (2.24±0.52 g).

Table 4 Incidence of strawberry anthracnose in petiole DI, petiole DR, average individual plant crown rot scale and crown rot rate by *C. gloeosporioides* inoculation and different kinds of phenolic acid pretreatment in pot experiment

Treatment	Disease index			
	Petiole DI	Petiole DR (%)	Average individual plant crown rot scale	Rot rate (%)
LF	0c	0	0c	0
LFC	34.88±15. 89a	40.38	1.25±0.70a	66.67
LP	0c	0	0c	0
LPC	43.91±20.11a	44.23	1.75±0.87a	66.67
HF	0c	0	0c	0
HFC	21.17±10.72b	23.72	0.58±0.44b	41.67
HP	0c	0	0	0
HPC	21.32±11.02b	28.12	0.53±0.34b	41.67
LT	0c	0	0c	0
LTC	1.66±1.77c	5.35	0.08±0.14c	8.33
CK	0c	0	0c	0
CKC	18.35±10.64b	27.11	0.42±0.25b	41.67

The data represent mean±SE (n=12). Values in the same row followed by the same level case letter are not statistically different at P=0.05 by Duncan's test

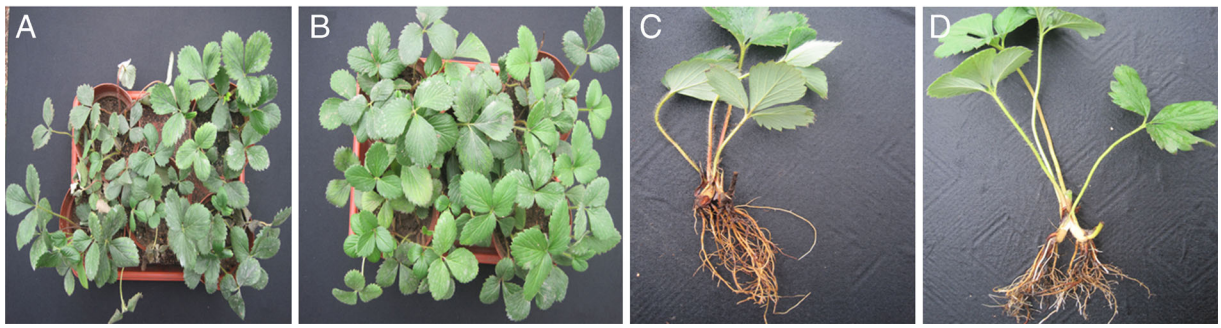

Fig. 5 The visual symptoms of strawberry anthracnose and normal strawberry plants. **a**: The symptoms of leaves wilt infected by strawberry anthracnose; **b**: normal growth strawberry plants; **c**: The symptoms of longitudinal sections of the main stem infected by strawberry anthracnose crown rot; **d**: The longitudinal sections of normal growth strawberry plants

Discussion

Root exudates represent the largest source of allelochemicals in rhizosphere soil (Jilani et al. 2008). In a previous study, Kitazawa et al. (2005) found that exudates (lactic, succinic, adipic, benzoic and *p*-hydroxybenzoic acid) from strawberry roots grown hydroponically significantly inhibited the fresh weights and dry weights of shoots and roots, and the maximum root length. Asao et al. (2008) and Asaduzzaman et al. (2012) exploited electro-degradation of root exudates to mitigate autotoxicity in hydroponically grown strawberry plants to recover their growth and yield. In the present study, our results demonstrated the presence of 10 phenolic acids in the rhizosphere soil of strawberry plants. Only *p*-hydroxybenzoic was found in previous studies, potentially due to different varieties, different cultural conditions and different methods of phenolic acid separation.

In the present study, the rhizosphere soil, plow layer soil and decomposed strawberry plants contained relatively high amounts of PA and FA. The chromatographic peak of TA in the rhizosphere soil sample was wider than the peak of TA in the standard sample chromatogram (Fig. 1). We speculate that the chromatographic peak of the retention time of 19.076 min contains TA, but it may be mixed with other materials that failed to separate. Therefore, some limitations may exist in calculating the content of TA based on the chromatographic peak area.

Negative plant–soil feedback plays an important role in soil sickness, which is one of the factors limiting the sustainable development of intensive agriculture (Huang et al. 2013). The effects of allelochemicals depend on their concentrations (Inderjit and Mallik 1996). Low concentrations of allelochemicals are generally believed to boost plant growth, but high concentrations can inhibit, promote or have no effect on plant

Table 5 Effects of adding different kinds and concentration of phenolic on total root length (cm), root surface area (cm^2), average root diameter (mm), root tips and root volume (cm^3) of strawberry plant

Treatment	Total root length (cm)	Root surface area (cm^2)	Average root diameter (mm)	Root tips volume (cm^3)	Root
LF	2113±395a	217±4 3 a	0.35±0.02 a	4418±1128 a	1.80±0.39 a
LP	2173±290a	194±27 a	0.32±0.04 a	4582±1301 a	1.39±0.21 a
HF	375±147b	47±19 b	0.45±0.04 b	864±265 b	0.50±0.26 b
HP	1977±493a	211±51 a	0.37±0.04 a	4404±1488 a	1.69±0.21 a
LT	2183±590a	202±51a	0.35±0.06 a	5107±1495 a	1.72±0.35 a
CK	2030±448a	191±47 a	0.34±0.02a	4516±1251 a	1.44±0.41a

The data represent mean±SE (n=12). Values in the same Column followed by the same level case letter are not statistically different at P= 0.05 by Duncan's test

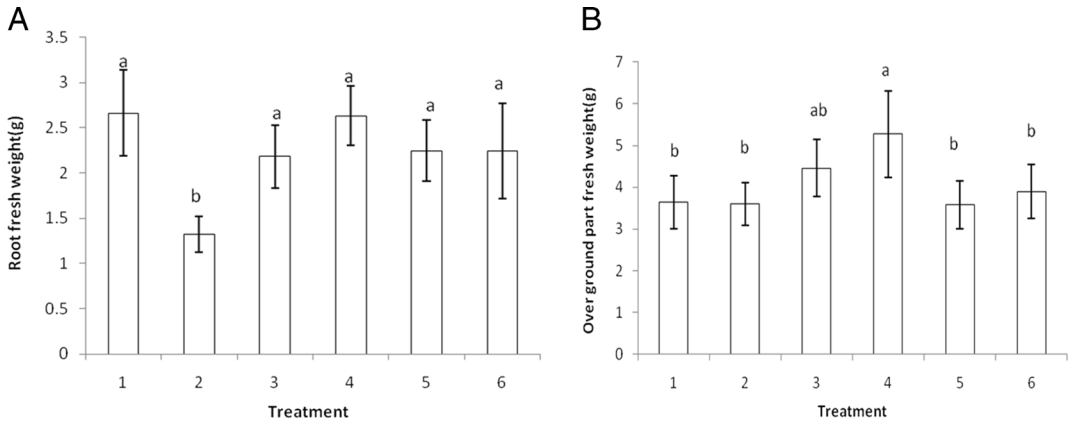

Fig. 6 Effects of different phenolic acids at varying concentrations on ground fresh weight and root fresh weight. (1) LF, (2) HF, (3) LP (4) LT, (5) HP, (6) CK. *Bars* indicate 2 times standard error. Different letters suggest significant difference among treatments by using ANOVA analysis at $p=0.05$

growth (Fries et al. 1997). In our study, we imposed a wide range of phenolic acid concentrations to identify allelopathic effects on *C. gloeosporioides*. Our research found that different kinds and concentrations of exogenous phenolic acids had different effects on colony growth (Table 3) and conidial germination of *C. gloeosporioides* (Figs. 2, 3 and 4). PA, which was present in the highest concentration in the plow layer and decomposed strawberry plants, significantly promoted colony growth at a low concentration (50 mg l^{-1}) and significantly inhibited growth at a high concentration (>200 mg l^{-1}), although significant ($P<0.05$) inhibition occurred on the conidial germination of *C. gloeosporioides* at low concentrations (25 and 50 mg l^{-1}; Fig. 2). Conidial germination was inhibited at a low concentration of PA (25 mg l^{-1}) and inhibition was gradually enhanced as the PA concentration increased (Fig. 2). The inhibition of conidial germination by PA coincides with the previously reported inhibition of conidial germination of *F. oxysporum* f.sp. *niveum* by salicylic acid (Wu et al. 2008a, b). Enhanced conidial germination of *C. gloeosporioides* was observed with an increasing FA concentration up to 50 mg l^{-1}. When the concentration of FA was greater than 50 mg l^{-1}, inhibition of conidial germination was very intense and actually increased with increasing FA concentrations (Fig. 2). The stimulation of conidial germination at a low FA concentration and the inhibition at high FA concentration are consistent with the reported stimulation or inhibition of conidial germination by sinapic acid on *F. oxysporum* f. sp. *niveum* (Wu et al. 2009a). In addition, chlorogenic acid significantly promoted the conidial germination of *C. gloeosporioides* at a low concentration (<200 mg l^{-1}; Fig. 3), while *trans*-cinnamic acid significantly inhibited conidial germination (Fig. 4) and growth (Table 1) at all trial concentrations.

We also tested for a low concentration of FA (25 μg g^{-1} dry soil), a high concentration of FA (100 μg g^{-1} dry soil), a low concentration of PA (25 μg g^{-1} dry soil), a high concentration of PA (100 μg g^{-1} dry soil) and a low concentration of TA (25 μg g^{-1} dry soil). Our results clearly showed that FA and PA at low concentrations (25 μg g^{-1} dry soil) significantly increased the petiole DI and crown rot scale of strawberry plants (Table 4). FA and PA at a high concentration (100 μg g^{-1} dry soil) were not different from the treatment of CKC in promoting strawberry anthracnose (Table 4), indicating that FA and PA at high concentrations (>100 μg g^{-1} dry soil) may alleviate anthracnose on strawberries grown in pots. FA and PA at a high concentration in vitro also inhibits colony growth (Table 3) and spore germination (Fig. 2) of *C. gloeosporioide*s. The effects of FA and PA on *C. gloeosporioides* is concentration-dependent, which agree with the potential for hormesis to occur in nature of (±)-catechin (Prithiviraj et al. 2007). However, FA at a high concentration (100 μg g^{-1} dry soil) can significantly limit the root growth (Table 5) and fresh weight (Fig. 5a) compared with the CK, HP and low concentrations (25 μg g^{-1} dry soil) of the remaining phenolic acids. *C. gloeosporioides* was more susceptible than the strawberry plant root to concentrations of phenolic acids. These results were consistent with that direct phytotoxic effects of phenolics on cucumber probably did not happen in continuous mono-cropping systems,

but they might indirectly influence cucumber performance by changing soil microbial communitie (Zhou et al. 2012b). A low concentration of TA (25 μg g^{-1} dry soil) can significantly limit colony growth (Table 3), conidial germination (Fig. 4), and the strawberry anthracnose crown rot in pots (Table 4), but with no effect on root structure (Table 5), fresh root weight (Fig. 5a) and above-ground growth of strawberry (Fig. 5b). TA promoted the incidence of Fusarium wilt in cucumber (Ye et al. 2004) and Fusarium wilt in watermelon (Wu et al. 2008a, b) and inhibited strawberry anthracnose crown rot, which indicate that the same phenolic acid have different effects on different kinds of pathogens.

In conclusion, our findings showed that diverse phenolic acids in plow soil had different influence on strawberry anthracnose crown rot. The effects of phenolic acids were concentration-dependent and *C. gloeosporioides* was more sensitive to phenolic acids concentration than root. Phenolic acids can be regulated to remit the occurrence of strawberry anthracnose crown rot.

Autotoxins influence soil microbes and lead to increased or decreased soil sickness (Huang et al. 2013). Intercropping with aerobic rice (*Oryza sativa* L.) suppressed Fusarium wilt in watermelon through the production of rice root exudates (Ren et al. 2008). Different plant root exudates or residues produce different allelochemicals under different conditions. The main allelochemicals produced by some plants, such as cucumber (Yu and Matsui 1994; Ye et al. 2004; Yu et al. 2009), eggplant (*Solanum melongena*) (Chen et al. 2011a, b) and pea (*Pisum sativum*) (Yu and Yoshihisa 1999) was TA. These results provide a possibility in preventing strawberry anthracnose crown rot by proper rotation, intercropping, proper soil and plant residue management.

Application of organic amendments has been proposed as a strategy to manage diseases caused by soilborne pathogens, but inconsistent results hinder their use (Bonanomi et al. 2010). Crop residues or green manure are important sources of allelochemicals, particularly the phenolic acids in the soil. Different types of crop residues or green manure produce different phenolic acids. Our study showed that different types and concentrations of phenolic acids have different effects on strawberry anthracnose crown rot, which may explain why the use of different types of organic material to control soilborne pathogens often achieve inconsistent results. The effects were found to depend on the particular organic material and soilborne pathogens.

Baker and Cook (1974) were the first to suggest the concept of disease suppression in soil, which was classified as general or specific disease suppression (Deacon 1984). Disease suppressive soil is widely distributed, but the mechanism of resistance to soilborne pathogens and application in sustainable agricultural development is not well understood. Allelopathy plays a significant role in sustainable agriculture (Chou 1999). Root exudates of many plants also contain antimicrobial compounds, but research in this area has been minimal (Huang et al. 2013). Our studies have shown that the regulation of allelochemicals, especially phenolic acids, and their concentrations, may present a direct way to develop specific disease suppressive soil.

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References

Asaduzzaman, M., Kobayashi, Y., Isogami, K., Tokura, M., Tokumasa, K., & Asao, T. (2012). Growth and yield recovery in strawberry plants under autotoxicity through electrodegradation. *European Journal of Horticultural Science, 77*(2), 58–67.

Asao, T., Kitazawa, H., Ban, T., & Habibur Rahman Pramanik, M. (2008). Electrodegradation of root exudates to mitigate autotoxicity in hydroponically grown strawberry (*Fragaria* × *ananassa* Duch.) plants. *Hort Science, 43*(7), 2034–2038.

Atanasova-Penichon, V., Pons, S., Pinson-Gadais, L., Picot, A., Marchegay, G., Bonnin-Verdal, M.-N., et al. (2012). Chlorogenic acid and maize ear rot resistance: a dynamic study investigating Fusarium graminearum development, deoxynivalenol production, and phenolic acid accumulation. *Molecular Plant-Microbe Interactions, 25*(12), 1605–1616. doi:10.1094/mpmi-06-12-0153-r.

Baker, K. F., & Cook, R. J. (1974). Biological control of plant pathogens (biological control of plant pathogens).

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science, 17*(8), 478–486. doi:10.1016/j.tplants.2012.04.001.

Blum, U., & Shafer, S. R. (1988). Microbial-populations and phenolic-acids in soil. *Soil Biology & Biochemistry, 20*(6), 793–800. doi:10.1016/0038-0717(88)90084-3.

Bonanomi, G., Antignani, V., Capodilupo, M., & Scala, F. (2010). Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology and Biochemistry, 42*(2), 136–144. doi:10.1016/j.soilbio.2009.10.012.

Boutigny, A.-L., Atanasova-Penichon, V., Benet, M., Barreau, C., & Richard-Forget, F. (2010). Natural phenolic acids from wheat bran inhibit Fusarium culmorum trichothecene biosynthesis in vitro by repressing Tri gene expression. *European Journal of Plant Pathology, 127*(2), 275–286. doi:10.1007/s10658-010-9592-2.

Cao, K. Q., & Wang, S. T. (2007). Autotoxicity and soil sickness of strawberry (Fragaria x ananassa). *Allelopathy Journal, 20*(1), 103–113.

Chen, L., Yang, X., Raza, W., Li, J., Liu, Y., Qiu, M., et al. (2011a). Trichoderma harzianum SQR-T037 rapidly degrades allelochemicals in rhizospheres of continuously cropped cucumbers. *Applied Microbiology and Biotechnology, 89*(5), 1653–1663. doi:10.1007/s00253-010-2948-x.

Chen, S., Zhou, B., Lin, S., Li, X., & Ye, X. (2011b). Accumulation of cinnamic acid and vanillin in eggplant root exudates and the relationship with continuous cropping obstacle. *African Journal of Biotechnology, 10*(14), 2659–2665.

Chou, C. H. (1999). Roles of allelopathy in plant biodiversity and sustainable agriculture. *Critical Reviews in Plant Sciences, 18*(5), 609–636. doi:10.1016/s0735-2689(99)00393-7.

Dalton, B.R., Weed, S.B. & Blum, U. (1987). Plant phenolic acids in soils: a comparison of extraction procedures. *Soil Science Society of America Journal, 51*, 1515–1521.

Deacon, J. W. (1984). The nature and practice of biological-control of plant-pathogens - cook, rj, baker, kf. *Nature, 309*(5970), 732. doi:10.1038/309732a0.

Delp, B. R., & Milholland, R. D. (1980). Evaluating strawberry fragaria-ananassa plants for resistance to colletotrichum-fragariae. *Plant Disease, 64*(12), 1071–1073.

Freeman, S., Shalev, Z., & Katan, J. (2002). Survival in soil of Colletotrichum acutatum and C-gloeosporioides pathogenic on strawberry. *Plant Disease, 86*(9), 965–970. doi:10.1094/pdis.2002.86.9.965.

Fries, L. L. M., Pacovsky, R. S., Safir, G. R., & Siqueira, J. O. (1997). Plant growth and arbuscular mycorrhizal fungal colonization affected by exogenously applied phenolic compounds. *Journal of Chemical Ecology, 23*(7), 1755–1767.

Garrido, C., Carbú, M., Fernández-Acero, F. J., Budge, G., Vallejo, I., Colyer, A., et al. (2007). Isolation and pathogenicity of Colletotrichum spp. causing anthracnose of strawberry in south west Spain. *European Journal of Plant Pathology, 120*(4), 409–415. doi:10.1007/s10658-007-9224-7.

Hao, W.-y., Ren, L.-x., Ran, W., & Shen, Q.-r. (2010). Allelopathic effects of root exudates from watermelon and rice plants on Fusarium oxysporum f.sp. niveum. *Plant and Soil, 336*(1–2), 485–497. doi:10.1007/s11104-010-0505-0.

He, C. N., Gao, W. W., Yang, J. X., Bi, W., Zhang, X. S., & Zhao, Y. J. (2008). Identification of autotoxic compounds from fibrous roots of Panax quinquefolium L. *Plant and Soil, 318*(1–2), 63–72. doi:10.1007/s11104-008-9817-8.

Huang, X. X., Bie, Z. L., & Huang, Y. (2010). Identification of autotoxins in rhizosphere soils under the continuous cropping of cowpea. *Allelopathy Journal, 25*(2), 383–392.

Huang, L. F., Song, L. X., Xia, X. J., Mao, W. H., Shi, K., Zhou, Y. H., et al. (2013). Plant-soil feedbacks and soil sickness: from mechanisms to application in agriculture. *Journal of Chemical Ecology, 39*(2), 232–242. doi:10.1007/s10886-013-0244-9.

Inderjit, & Mallik, A. U. (1996). The nature of interference potential of Kalmia angustifolia. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 26*(11), 1899–1904.

Jilani, G., Mahmood, S., Chaudhry, A. N., Hassan, I., & Akram, M. (2008). Allelochemicals: sources, toxicity and microbial transformation in soil - a review. *Annals of Microbiology, 58*(3), 351–357.

Kitazawa, H., Asao, T., Ban, T., Pramanik, M. H. R., & Hosoki, T. (2005). Autotoxicity of root exudates from strawberry in hydroponic culture. *Journal of Horticultural Science & Biotechnology, 80*(6), 677–680.

Kong, C. H., Wang, P., Zhao, H., Xu, X. H., & Zhu, Y. D. (2008). Impact of allelochemical exuded from allelopathic rice on soil microbial community. *Soil Biology and Biochemistry, 40*(7), 1862–1869. doi:10.1016/j.soilbio.2008.03.009.

Larry, L. S. (2008). *Integrated Pest Management for Strawberries*. California: UC IPM.

Lorenzo, P., Pereira, C. S., & Rodríguez-Echeverría, S. (2013). Differential impact on soil microbes of allelopathic compounds released by the invasive Acacia dealbata Link. *Soil Biology and Biochemistry, 57*, 156–163. doi:10.1016/j.soilbio.2012.08.018.

MacKenzie, S. J., Legard, D. E., Timmer, L. W., Chandler, C. K., & Peres, N. A. (2006). Resistance of strawberry cultivars to crown rot caused by Colletotrichum gloeosporioides isolates from Florida is nonspecific. [Article]. *Plant Disease, 90*(8), 1091–1097. doi:10.1094/pd-90-1091.

Nazih, N., Finlay-Moore, O., Hartel, P. G., & Fuhrmann, J. J. (2001). Whole soil fatty acid methyl ester (FAME) profiles of early soybean rhizosphere as affected by temperature and matric water potential. *Soil Biology & Biochemistry, 33*(4–5), 693–696. doi:10.1016/s0038-0717(00)00197-8.

Nemec, S. (1976). Response of three root rot fungi to strawberry phenolics and the relation of phenolics to disease resistance. *Mycopathologia, 59*(1), 37–40. doi:10.1007/BF00491202.

Prithiviraj, B., Perry, L. G., Badri, D. V., & Vivanco, J. M. (2007). Chemical facilitation and induced pathogen resistance mediated by a root-secreted phytotoxin. *The New Phytologist, 173*(4), 852–860. doi:10.1111/j.1469-8137.2006.01964.x.

Ren, L., Su, S., Yang, X., Xu, Y., Huang, Q., & Shen, Q. (2008). Intercropping with aerobic rice suppressed Fusarium wilt in watermelon. *Soil Biology and Biochemistry, 40*(3), 834–844. doi:10.1016/j.soilbio.2007.11.003.

Salazar, S. M., Castagnaro, A. P., Arias, M. E., Chalfoun, N., Tonello, U., & Ricci, J. C. D. (2007). Induction of a defense response in strawberry mediated by an avirulent strain of Colletotrichum. *European Journal of Plant Pathology, 117*(2), 109–122. doi:10.1007/s10658-006-9075-7.

Shi, S., Richardson, A. E., O'Callaghan, M., DeAngelis, K. M., Jones, E. E., Stewart, A., et al. (2011). Effects of selected root

exudate components on soil bacterial communities. *FEMS Microbiology Ecology, 77*(3), 600–610. doi:10.1111/j.1574-6941.2011.01150.x.

Weir, T. L., Park, S. W., & Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology, 7*(4), 472–479. doi:10.1016/j.pbi.2004.05.007.

Widiastuti, A., Yoshino, M., Saito, H., Maejima, K., Zhou, S. Y., Odani, H., et al. (2013). Heat shock-induced resistance in strawberry against crown rot fungus Colletotrichum gloeosporioides. [Article]. *Physiological and Molecular Plant Pathology, 84*, 86–91. doi:10.1016/j.pmpp.2013.08.003.

Wu, H.-S., Raza, W., Fan, J.-Q., Sun, Y.-G., Bao, W., & Shen, Q.-R. (2008a). Cinnamic acid inhibits growth but stimulates production of pathogenesis factors by in vitro cultures of Fusarium oxysporum f.sp niveum. *Journal of Agricultural and Food Chemistry, 56*(4), 1316–1321. doi:10.1021/jf0726482.

Wu, H. S., Raza, W., Fan, J. Q., Sun, Y. G., Bao, W., Liu, D. Y., et al. (2008b). Antibiotic effect of exogenously applied salicylic acid on in vitro soilborne pathogen, Fusarium oxysporum f.sp.niveum. *Chemosphere, 74*(1), 45–50. doi:10.1016/j.chemosphere.2008.09.027.

Wu, H.-s., Wang, Y., Bao, W., Liu, D.-y., Raza, W., Huang, Q.-w., et al. (2009a). Responses of Fusarium oxysporum f. sp. niveum to exogenously added sinapic acid in vitro. *Biology and Fertility of Soils, 45*(4), 443–447. doi:10.1007/s00374-009-0353-3.

Wu, H.-S., Wang, Y., Zhang, C.-Y., Bao, W., Ling, N., Liu, D.-Y., et al. (2009b). Growth of in vitro Fusarium oxysporum f. sp niveum in chemically defined media amended with gallic acid. *Biological Research, 42*(3), 297–304.

Wu, H.-s., Luo, J., Raza, W., Liu, Y.-x., Gu, M., Chen, G., et al. (2010a). Effect of exogenously added ferulic acid on in vitro Fusarium oxysporum f. sp. niveum. *Scientia Horticulturae, 124*(4), 448–453. doi:10.1016/j.scienta.2010.02.007.

Wu, H. S., Liu, Y. D., Yang, X. I., Chen, X. Q., Wang, Z. H., Kong, X. Y., et al. (2010b). Growth responses of in vitro Fusarium oxysporum f. sp. niveum to external supply of tannic acid. *Journal of Environmental Biology, 31*(6), 1017–1022.

Xu, Y., Wang, G., Jin, J., Liu, J., Zhang, Q., & Liu, X. (2009). Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. *Soil Biology and Biochemistry, 41*(5), 919–925. doi:10.1016/j.soilbio.2008.10.027.

Ye, S. F., Yu, J. Q., Peng, Y. H., Zheng, J. H., & Zou, L. Y. (2004). Incidence of Fusarium wilt in Cucumis sativus L. is promoted by cinnamic acid, an autotoxin in root exudates. *Plant and Soil, 263*(1–2), 143–150. doi:10.1023/B:Plso.0000047721.78555.Dc.

Yu, J. Q., & Matsui, Y. (1994). Phytotoxic substances in root exudates of cucumber (Cucumis-sativus l). *Journal of Chemical Ecology, 20*(1), 21–31. doi:10.1007/bf02065988.

Yu, J., & Yoshihisa, M. (1999). Autointoxication of root exudates in Pisum sativus. *Acta Horticulturae Sinica, 26*(3), 175–179.

Yu, J., Sun, Y., Zhang, Y., Ding, J., Xia, X., Xiao, C., et al. (2009). Selective trans-cinnamic acid uptake impairs [Ca2+]cyt homeostasis and growth in Cucumis sativus L. *Journal of Chemical Ecology, 35*(12), 1471–1477. doi:10.1007/s10886-009-9726-1.

Zhao, X., Zhen, W., Qi, Y., Liu, X., & Yin, B. (2009). Coordinated effects of root autotoxic substances and Fusarium oxysporum Schl. f. sp. fragariae on the growth and replant disease of strawberry. *Frontiers of Agriculture in China, 3*(1), 34–39. doi:10.1007/s11703-009-0006-1.

Zhou, X., & Wu, F. (2012a). Effects of amendments of ferulic acid on soil microbial communities in the rhizosphere of cucumber (Cucumis sativus L.). *European Journal of Soil Biology, 50*, 191–197. doi:10.1016/j.ejsobi.2012.03.001.

Zhou, X., & Wu, F. (2012b). p-Coumaric acid influenced cucumber rhizosphere soil microbial communities and the growth of Fusarium oxysporum f.sp. cucumerinum Owen. *PloS One, 7*(10), e48288. doi:10.1371/journal.pone.0048288.

Zhou, X., & Wu, F. (2013). Artificially applied vanillic acid changed soil microbial communities in the rhizosphere of cucumber (Cucumis sativusL.). *Canadian Journal of Soil Science, 93*(1), 13–21. doi:10.4141/cjss2012-039.

Zhou, X., Yu, G., & Wu, F. (2012a). Responses of soil microbial communities in the rhizosphere of cucumber (Cucumis sativus L.) to exogenously applied p-hydroxybenzoic acid. *Journal of Chemical Ecology, 38*(8), 975–983. doi:10.1007/s10886-012-0156-0.

Zhou, X., Yu, G., & Wu, F. (2012b). Soil phenolics in a continuously mono-cropped cucumber (Cucumis sativus L.) system and their effects on cucumber seedling growth and soil microbial communities. *European Journal of Soil Science, 63*(3), 332–340. doi:10.1111/j.1365-2389.2012.01442.x.