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ARTICLE

Effects of ferulic and *p*-hydroxybenzoic acids on *Fusarium* community structure and abundance in cucumber seedling rhizosphere

Huilin Xie and Xingang Zhou

Abstract: Soil microorganisms play an important role in agricultural ecosystem. However, there is little information about the effects of putative allelochemicals on specific soil microorganisms in vivo. Cucumber seedlings were treated with four concentrations of ferulic and *p*-hydroxybenzoic acids (0–1.0 μmol·g^{-1} soil) in soil. Effects of ferulic and *p*-hydroxybenzoic acids on rhizosphere *Fusarium* community structures and abundance were analyzed by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) and real-time PCR techniques, respectively. The results showed that ferulic acid at concentrations of 0.25, 0.5, and 1.0 μmol·g^{-1} soil significantly reduced the number of bands of *Fusarium*, and ferulic acid at concentrations of 0.5 and 1.0 μmol·g^{-1} soil significantly reduced Shannon–Wiener and evenness index of *Fusarium* community. All concentrations of *p*-hydroxybenzoic acid changed the community structure of *Fusarium*, including decreasing the number of bands, Shannon–Wiener, and evenness index. Ferulic and *p*-hydroxybenzoic acids at all concentrations significantly promoted the abundance of *Fusarium*. Ferulic and *p*-hydroxybenzoic acids at 0.5 μmol·g^{-1} soil had the highest *Fusarium* abundance among all treatments. These results indicate that four concentrations of ferulic acid had various effects on *Fusarium*, which was different from *p*-hydroxybenzoic acid, and this may be related to the cucumber autotoxicity, giving us a further understanding of soil sickness.

Key words: autotoxicity, *Fusarium*, soil microbial community, ferulic acid, *p*-hydroxybenzoic acid.

Résumé : Les microorganismes du sol jouent un rôle important dans l'écosystème agricole. Cependant, on sait peu de choses concernant les effets des substances allélochimiques putatives sur certains microorganismes in vivo. Les auteurs ont traité des plantules de concombre avec de l'acide férulique et de l'acide *p*-hydroxybenzoïque à quatre concentrations (0-1,0 μmol·g^{-1} de sol) dans le sol. Ensuite ils ont analysé les effets de ces deux acides sur la structure communautaire et l'abondance des cryptogames du genre *Fusarium* dans la rhizosphère, respectivement par la réaction en chaîne de la polymérase couplée à l'électrophorèse sur gel en gradient dénaturant (PCR-DGGE) et par la PCR en temps réel. Selon les résultats obtenus, une concentration d'acide férulique de 0,25, 0,5 ou 1,0 μmol·g^{-1} de sol réduit sensiblement le nombre de bandes correspondant à *Fusarium*, tandis que l'acide férulique réduit sensiblement l'indice de Shannon et l'indice d'homogénéité de la population fusarienne aux concentrations de 0,5 et 1,0 μmol·g^{-1} de sol. L'acide *p*-hydroxybenzoïque modifie la structure communautaire de *Fusarium* à toutes les concentrations, notamment en diminuant le nombre de bandes, l'indice de Shannon et l'indice d'homogénéité. Peu importe leur concentration, les deux acides accroissent significativement l'abondance du cryptogame. À 0,5 μmol·g^{-1} de sol, ils engendrent tous deux la plus grande abondance de *Fusarium* parmi tous les traitements. Ces résultats laissent croire que l'effet de l'acide férulique sur *Fusarium* varie avec la concentration et diffère des effets attribuables à l'acide *p*-hydroxybenzoïque. On pourrait le devoir à l'autotoxicité du concombre, ce qui nous éclaire un peu mieux sur la fatigue des sols. [Traduit par la Rédaction]

Mots-clés : autotoxicité, *Fusarium*, microflore du sol, acide férulique, acide *p*-hydroxybenzoïque.

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Introduction

In agro-ecosystems, the decline in crop yield and quality resulting from continuous monoculture on the same land is referred to as soil sickness (Bever et al. 2012; Muzell Trezzi et al. 2016; Zhou et al. 2018). The possible factors leading to soil sickness include accumulation of soil-borne pathogens, deterioration of soil physiochemical properties, and accumulation of allelochemicals (Yu et al. 2000; Zhou et al. 2012). Phenolic acids are secondary metabolites in plants, which are considered to be the main factor leading to soil sickness (Sun et al. 2013). Many phenolic acids have also been found in plants, root exudates, and soil as autotoxins (Huang et al. 2013), And these acids enter soil affect crop growth and the abundance and community composition of plant-pathogenic microorganisms (Yu et al. 2003; Lee et al. 2006). Recent in vitro studies have shown that phenolic acids can affect the growth and physiological status of specific microorganisms in soil, such as *Fusarium* and *Trichoderma* (Zhou et al. 2018). However, studies have shown that different phenolic acids have varied impacts on the same soil microorganisms (Liu et al. 2016).

It was found that *Fusarium* community size was related to soil sickness associated with cucumber planting. *Fusarium* is a genus of filamentous fungi that contains many agronomically important plant pathogens, its pathogenic strains can cause vascular diseases of plants, and also harm crops such as watermelon, cucumber, tomato, pepper, melon, soybean, and cotton all the year round (Armstrong and Armstrong 1981; Gordon and Martyn 1997; Nelson and Horts 1983; McKeen and Wensley 1961). They destroy host plants by invading host vascular tissues through mycelia, secreting hydrolases, producing mycotoxins and apoptosis of host plants cells during infection (Booth 1972; Pavlovkin et al. 2004; Wu et al. 2010). There are many species of *Fusarium* including *Fusarium oxysporum*, *F. equiseti*, *F. solani*, *F. moniliforme*, and *F. proliferatum* (Chen et al. 2018). Among them, *Fusarium oxysporum* f.sp. *cucumerinum* is the most important soil-borne pathogen, which severely limits cucumber yield and affects the growth of plant in soil (Ahn et al. 1998).

In the soil–microbiome–plant system, the soil microbial community is considered a key to maintain soil health (Van Der Heijden et al. 2008; Bardgett and Van Der Putten 2014). Many studies have shown that plant autotoxins cause soil sickness by negatively affecting the diversity and abundance of rhizosphere soil microbial communities (Liu et al. 2016). Cucumber is continuously monocultured in greenhouse all year round, accounting for more than 40% of vegetable production area in China (Yu et al. 2000; Zhou et al. 2017). However, the production of cucumber is severely restricted by soil sickness. Previous studies have found that cucumber is susceptible to phenolic acid during the seedling stage and the main phenolic acids in cucumber-cultivated soil, including ferulic and *p*-hydroxybenzoic acids (Zhou et al. 2012). Previously, we reported that both ferulic and *p*-hydroxybenzoic acids inhibited the growth of cucumber seedlings and changed the compositions of bacterial and fungal communities (Jin et al. 2020). On the other hand, we reported that various concentrations of exogenous phenolic acid (syringic acid, vanillic acid) had different effects on *Fusarium* community (Zhang et al. 2018; Chen et al. 2018). However, the effects of ferulic and *p*-hydroxybenzoic acids on the community structures and abundance of *Fusarium* are still unclear. Therefore, the purpose of this study was to investigate the effects of exogenous ferulic and *p*-hydroxybenzoic acids on the structures and abundance of *Fusarium* communities in cucumber rhizosphere.

Materials and Methods

Greenhouse experiment

Cucumber seedlings (cv. Jinlu 3) were soaked in water at 55 °C for 30 min, and then germinated in sand in a grow chamber at 26 °C, then we transplanted cucumbers with two cotyledons into a pot with a diameter of 10 cm, a height of 10 cm, and containing 150 g soil (one plant per pot). The soil was collected from an open field in our Horticulture Experimental Station (45°41′N, 126°37′E; mean altitude, 127.95 m; annual precipitation, 524.5 mm; maximum and minimum temperature, 36.7 °C and −37.7 °C) of Northeast Agricultural University in Harbin that was covered with grasses and undisturbed since more than 15 years, contained organic matter 3.67%, available nitrogen 89.02 $mg \cdot kg^{-1}$, available phosphorus 63.36 $mg \cdot kg^{-1}$, available potassium 119.15 $mg \cdot kg^{-1}$, EC (1:2.5, *w*/*v*) 0.33 $mS \cdot cm^{-1}$, pH (1:2.5, *w*/*v*) 7.78, and cucumber transplanted seedlings were grown in the greenhouse (32 °C during daytime and 22 °C at night; relative humidity, 60%–80%; 16 h light/8 h dark).

Previous studies have found that phenolic acids can be rapidly depleted after they are added to the soil due to the utilization of phenolic acids by microorganisms under favorable environmental conditions (Qu and Wang 2008). Therefore, in this study, when cucumber grew to one-leaf stage, four concentrations of both ferulic and *p*-hydroxybenzoic acids (0, 0.02, 0.05, 0.1, and 0.2 $\mu mol \cdot g^{-1}$ soil) were, respectively, applied to the seedlings every two days (once every 48 h) and received a final concentration of 0, 0.1, 0.25, 0.5, and 1 $\mu mol \cdot g^{-1}$ soil, respectively. About 0.5 mL of ferulic acid, *p*-hydroxybenzoic acid, or water solutions were regularly added on the soil surface by a syringe to maintain the required level, as described by Shafer and Blum (1991). Both ferulic and *p*-hydroxybenzoic acids were purchased from Solibol Life Sciences, Beijing, China. The pH of the solution was adjusted to 7.0 with 0.1 $mol \cdot L^{-1}$ NaOH solution. Seedlings treated with distilled water were used as control. To maintain a constant weight of pots, distilled water is used to adjust the soil water content every two days to keep the water content above 60% of the water holding capacity. In total, the final concentrations of W, T1, T2, T3, and T4 treatments were 0,

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0.1, 0.25, 0.5, and 1 μmol·g^{-1} soil, respectively. Each treatment had five pots and was replicated three times. The total duration of the greenhouse experiment was three weeks.

Rhizosphere soil sampling and DNA extraction

On the day after the fifth application of ferulic and *p*-hydroxybenzoic acids, as previously described, the cucumber seedlings were carefully taken out from the pots, and soils loosely attached to cucumber roots were charily removed by manual shaking. Soil closely attached to roots was considered as rhizosphere soil, which was removed from the surface of roots with sterile brush. Cucumber rhizosphere soil samples were collected from five plants of each treatment replicate, 3 g of rhizosphere soil was taken from each pot and mixed to obtain a composite sample, and cucumber rhizosphere soil samples were stored at –80 °C after sieving through a 2 mm mesh. Three composite samples were obtained for each treatment.

About 0.25 mg of rhizosphere soil DNA was extracted by using the Power Soil® DNA Isolation Kit (MO BIO Laboratories, CA, USA) according to the manufacturer's protocol. DNA was extracted from each composite soil sample for three times, and the extracted DNA solutions were pooled.

PCR-DGGE analysis

The community structure of *Fusarium* in soil was determined by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE). Nested PCR was used to amplify the *Fusarium Ef1cα* gene with primer sets of EF-1/EF-2 (O'Donnell et al. 1998) and Alfie1/Alfie2 (Yergeau et al. 2005) in the first and second round of PCR amplifications, respectively. The first and second round of PCR amplification, 50 μL PCR reaction system: template 3 μL; 10× Buffer 5 μL; Mg^{2+} 3 and 4 μL; dNTP 4 μL; 1 μL for each primer; rTaq enzyme 1 μL; deionized water 31 and 32 μL. Fifty nanograms of soil DNA or 3 μL of the first-strand cDNAs was used as template in the first-round PCR, and 3 μL of 10-fold diluted first-round PCR products was used as template in the second-round PCR. The PCR protocol was: 94 °C for 7 min; followed by 35 cycles of 94 °C for 60 s for *Ef1a* genes, 53 °C for 60 s for EF-1/EF-2 (67 °C for Alfie1- GC/Alfie2) and 72 °C for 60 s; and a final extension at 72 °C for 15 min. Each soil sample was amplified 3 times in parallel, and ddH_2O was used as template and as negative control.

Quantitative DGGE was performed using 6% (*w*/*v*) acrylamide gel to perform 40%–60% denaturation gradient on *Fusarium* (Wakelin et al. 2008) electrophoresis using DCode Universal mutation detection system (Bio-RAD Lab, LA, USA), After 14 h electrophoresis in 1xTAE (Tris-acetate-EDTA) buffer, the gel was stained in 1:3300 (*v*/*v*) GelRed (Biotium, CA, USA) nucleic acid staining solution for 20 min. DGGE maps were captured under UV light using AlphaImager HP imaging system (Alpha Innotech Crop., CA, USA).

Quantitative PCR

The abundance of *Fusarium* community was determined by IQ5 real-time PCR system (SYBR Green qPCR) of Bio-Rad Laboratory. The first round of PCR amplification system was same as PCR-DGGE analysis. The second round of PCR assays was conducted in a 20 μL volume containing 10 μL of 2× SYBR real-time PCR premixture (Sangon Biotech, China), 0.2 mmol·L^{-1} of each primer, 8 μg of BSA, and 3 μL 10-fold diluted first-round PCR products. The PCR conditions were as follows: (*i*) 94 °C for 5 min, (*ii*) 35 and 30 cycles of 94 °C for 45 s, 53 °C and 67 °C for 45 s, and 72 °C for 90 s for the first- and second-round amplification of the *Fusarium Ef1α* gene, respectively, and (*iii*) 72 °C for 10 min. Each soil sample was amplified 3 times in parallel, and ddH_2O was used as template as negative control. The relative *Fusarium* community abundance was calculated as described by Wakelin et al. (2008), all treatments were then compared with control soils. Sterile water was used as a negative control to replace templates, and all amplifications were performed in triplicate. The specificity of the products was confirmed by melting curve analysis and agarose gel electrophoresis. The threshold cycle (Ct) values obtained for each sample were compared with the standard curve to determine the target gene copy numbers of each sample.

Data analysis

The DGGE band was analyzed by Bio-Rad Quantity One software (version 4.5). Principal component analysis (PCA) was performed with Canoco for windows software (version 4.5) (Matsuyama et al. 2007). The position and intensity of each band were determined automatically. The density value of each band was divided by the average band density of the lane to minimize the influence of the loaded DNA concentrations among samples (Zhou et al. 2012). The number of DGGE bands, Shannon–Wiener index, and evenness index were calculated (Ran et al. 2021). Permutational analysis of variance (PERMANOVA) was performed to test the effects of phenolic acid on *Fusarium* community structure based on the Bray–Curtis distance dissimilarities. Analytical data and soil microbial abundance determined by qPCR were compared across treatments by one-way analysis of variance (ANOVA), and Tukey's honestly significant difference (HSD) test was used for average comparison at 0.05 probability level.

Results

Effects of ferulic acid on *Fusarium* community structure

The addition of ferulic acid resulted in significant changes in the DGGE profile of soil *Fusarium* except for the treatment of 0.1 μmol·g^{-1} soil ferulic acid compared to the control (Fig. 1*a*). PCA plot for the DGGE banding

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Fig. 1. PCR-DGGE profile (*a*) and its PCA analysis of *Fusarium* community treated with ferulic acid (*b*). **Note:** W represents control; T1, T2, T3, and T4 represent ferulic acid at the concentration of 0.1, 0.25, 0.5, 1.0 μmol·g^{-1} soil, respectively.

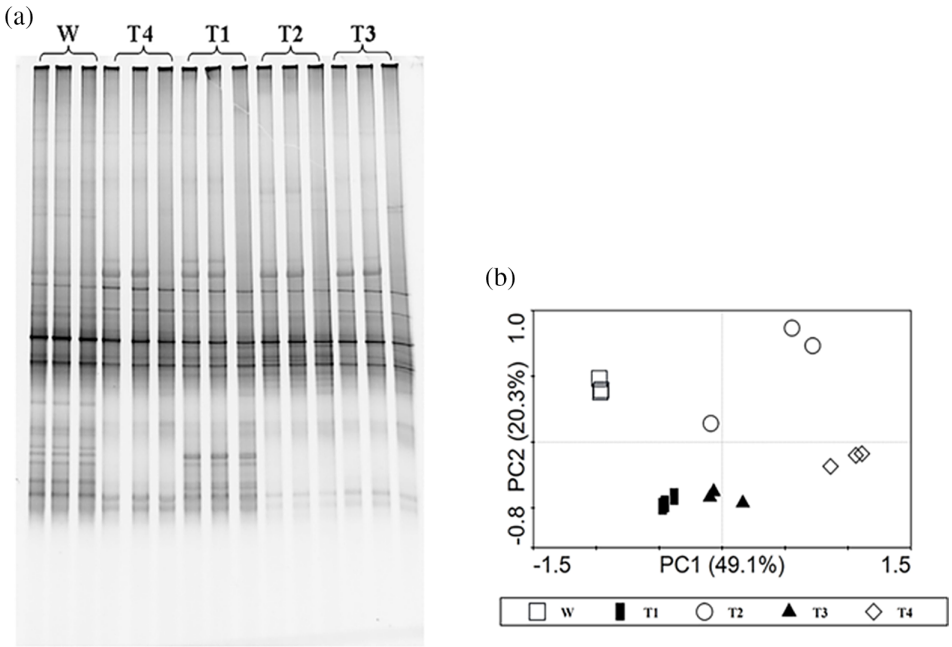

Table 1. Effects of ferulic and *p*-hydroxybenzoic acids on the number of visible bands (*S*) and Shannon–Wiener index (*H*) and evenness index (*E*) of *Fusarium* DGGE profile.

Concentration (μmol·g^{-1} soil)	Ferulic acid			*p*-Hydroxybenzoic acid		
	S	*H*	*E*	*S*	*H*	*E*
0	16.33 ± 0.58a	2.61 ± 0.08a	0.86 ± 0.03a	17.67 ± 0.58a	2.74 ± 0.05a	0.87 ± 0.02a
0.10	15.67 ± 0.58ab	2.69 ± 0.04a	0.88 ± 0.01a	11.67 ± 0.58bc	2.43 ± 0.05b	0.77 ± 0.02b
0.25	14.33 ± 0.58b	2.54 ± 0.03ab	0.83 ± 0.01a	12.00 ± 0.00bc	2.34 ± 0.01b	0.75 ± 0.00b
0.50	11.67 ± 0.58c	2.34 ± 0.02bc	0.77 ± 0.01b	12.33 ± 0.58b	2.33 ± 0.06b	0.74 ± 0.02b
1.00	11.00 ± 1.00c	2.28 ± 0.13c	0.75 ± 0.04b	10.67 ± 0.58c	2.17 ± 0.06c	0.69 ± 0.02c
ANOVA						
$F_{4,10}$	61	27.02	25.53	117.3	80.14	81.21
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note: 0.10, 0.25, 0.50, and 1.00 represent ferulic and *p*-hydroxybenzoic acid at the concentration of 0.1, 0.25, 0.5, 1.0 μmol·g^{-1} soil, respectively. 0 represents samples treated with water. Different letters indicate significant differences between treatments ($P < 0.05$, Tukey's HSD test).

pattern of *Fusarium* explained 49.1% and 20.3% of variations in the first two PCA axis, respectively. The separation between treatments can be seen in the PCA plot (Fig. 1*b*), indicating that the community structure of *Fusarium* was different in four treatments. PERMANOVA analysis also confirmed that ferulic acid significantly altered *Fusarium* community structure ($r^2 = 0.877$, $P < 0.01$).

Cucumber treated with 0.25, 0.5, 1.0 μmol·g^{-1} soil ferulic acid decreased the number of bands in a dose-dependent manner. In addition, Shannon–Wiener index and evenness index of 0.5 and 1.0 μmol·g^{-1} soil ferulic acid treatments were significantly lower than those of the control. In contrast, Shannon–Wiener index and evenness index of 0.25 μmol·g^{-1} soil ferulic acid treatment showed no difference compared with the control. There was no difference in *Fusarium* community structure between 1.0 μmol·g^{-1} soil ferulic acid treatment and control (Table 1).

Effects of *p*-hydroxybenzoic acid on *Fusarium* community structure

The DGGE analysis showed that the banding pattern of *Fusarium* was significantly different between control and other four treatments, and the banding pattern was consistent among the replicates of each treatment

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Fig. 2. PCR-DGGE profile (*a*) and its PCA analysis of *Fusarium* community treated with *p*-hydroxybenzoic acid (*b*). **Note**: W represents control; T1, T2, T3, and T4 represent *p*-hydroxybenzoic acid at the concentration of 0.1, 0.25, 0.5, 1.0 μmol·g^{-1} soil, respectively.

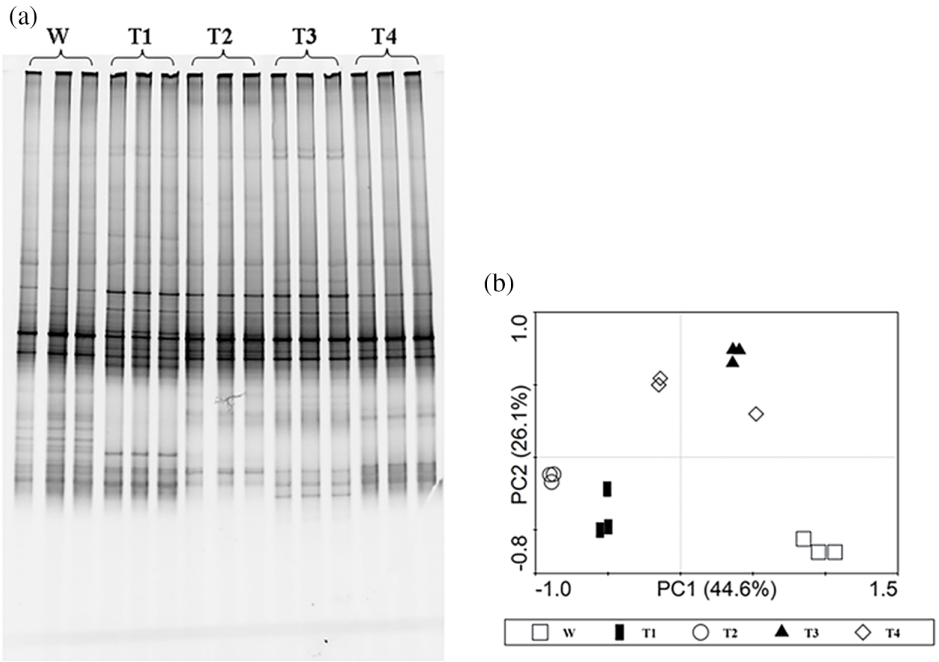

Table 2. Effects of ferulic and *p*-hydroxybenzoic acids at four concentrations on the abundance of *Fusarium* community in soil.

Concentration (μmol·g^{-1} soil)	Ferulic acid	*p*-Hydroxybenzoic acid
0	1.00 ± 0.12d	1.00 ± 0.12d
0.10	2.16 ± 0.08c	2.69 ± 0.41c
0.25	4.44 ± 0.45b	3.50 ± 0.24b
0.50	6.40 ± 0.28a	6.27 ± 0.23a
1.00	4.87 ± 0.23b	4.19 ± 0.30b
ANOVA		
$F_{4,10}$	201.69	148.21
P	<0.0001	<0.0001

Note: 0.10, 0.25, 0.50, and 1.00 represent ferulic and *p*-hydroxybenzoic acid at the concentration of 0.1, 0.25, 0.5, 1.0 μmol·g^{-1} soil, respectively. 0 represents samples treated with water. Different letters indicate significant differences between treatments ($P < 0.05$, Tukey's HSD test).

(Fig. 2*a*). All treatments can be clearly distinguished by PCA analysis of the DGGE profile of *Fusarium* (Fig. 2*b*). PC1 and PC2 explained 44.6% and 26.1% of the variations, respectively. PCA analysis showed that *p*-hydroxybenzoic acid treatment could be divided into two groups, treatment of 0.1 and 0.25 μmol·g^{-1} soil *p*-hydroxybenzoic acid were close to each other and treatment of 0.5 and 1.0 μmol·g^{-1} soil *p*-hydroxybenzoic acid were close to each other. These results indicated that significant differences in the structure of *Fusarium* in the other four treatments compared to the control. PERMANOVA analysis also confirmed that *p*-hydroxybenzoic acid significantly altered *Fusarium* community structure ($r^2 = 0.981$, $P < 0.01$).

The number of visible bands, Shannon–Wiener index, and evenness index of cucumber soil treated with 0.1–1.0 μmol·g^{-1} soil *p*-hydroxybenzoic acid were significantly lower than those treated with distilled water (Table 1). These diversity indices decreased by increasing *p*-hydroxybenzoic acid concentration.

Effects of ferulic and *p*-hydroxybenzoic acids on *Fusarium* community abundance

Quantitative PCR showed that all concentrations of ferulic and *p*-hydroxybenzoic acids significantly increased the community abundance of *Fusarium* in cucumber rhizosphere ($P < 0.05$) (Table 2). In the range of 0.1–0.5 μmol·g^{-1} soil, the abundance of *Fusarium* increased with the increase of ferulic acid concentration. The microbial community abundance of *Fusarium* was the highest under ferulic acid treatment of 0.5 μmol·g^{-1} soil, which was 4.87 times of the control. When the concentration of ferulic acid was higher than 1.0 μmol·g^{-1} soil, the abundance of *Fusarium* was lower than the 0.5 μmol·g^{-1} soil concentration, but it was still higher than the control. It was also similar to the treatment of *Fusarium* with 0.1–0.5 μmol·g^{-1} soil *p*-hydroxybenzoic acid. The abundance of *Fusarium* was the highest under the treatment of 0.5 μmol·g^{-1} concentration of *p*-hydroxybenzoic acid, which was 4.19 times of the control. When the concentration of *p*-hydroxybenzoic acid was higher than

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1.0 μmol·g^{-1} soil, the abundance of *Fusarium* was lower than the 0.5 μmol·g^{-1} soil concentration, but it was still higher than the control.

Discussion

In monoculture systems, the accumulation of autotoxins is considered to inhibit the growth of plants and affect soil microorganisms, leading to increased soil-borne diseases (Inderjit et al. 2011). The autotoxin isolated and identified from cucumber root exudates is mainly phenolic acids. Among them, benzoic acid, *p*-hydroxybenzoic acid, and other autotoxin played an important role in the interaction between cucumber plants and soil microorganisms, and changed soil microbial community (Zhou et al. 2012).

Previous studies showed that the effects of different phenolic compounds on *Fusarium* community structure and diversity were not consistent. For example, vanillin reduced the number of visible bands of *Fusarium* community, but did not affect Shannon–Wiener index and evenness index of *Fusarium* community structure (Zhou et al. 2018). Another study showed that vanillic acid (0.02 and 0.05 μmol·g^{-1} soil) increased the number of bands, Shannon–Wiener and evenness index of *Fusarium* (Chen et al. 2018). However, in this study, high concentration of ferulic acid significantly reduced the visible band number, Shannon–Wiener index and evenness index, and changed the community structure of *Fusarium*. Low concentration of ferulic acid had no significant effect on the community structure of *Fusarium*. In contrast, all concentrations of *p*-hydroxybenzoic acid changed the *Fusarium* community structure. Therefore, different phenolic acids may play different roles in plant–rhizosphere microbial interactions.

Quantitative PCR showed that ferulic and *p*-hydroxybenzoic acids could stimulate the proliferation of *Fusarium* in cucumber rhizosphere. In an earlier study, we reported that vanillin at a concentration of 0.02–0.2 μmol·g^{-1} soil promoted *Fusarium* abundance in soil (Zhou et al. 2018). The abundance of *Fusarium* in soil increased significantly after the addition of syringic acid, suggesting that *Fusarium* could use phenolic acid to promote its growth (Zhang et al. 2018). Chen et al. (2018) found that low vanillic acid concentrations (0.02–0.05 μmol·g^{-1} soil) increased *Fusarium* abundance, but high vanillic acid concentrations (0.1–0.2 μmol·g^{-1} soil) had the opposite effect. This study further confirmed that soil microbial communities can utilize phenolic acids as an effective carbon source (Badri et al. 2013).

Although ferulic and *p*-hydroxybenzoic acids increased the abundance of *Fusarium*, they inhibited the number of DGGE bands, Shannon–Wiener index index, and evenness index of *Fusarium* to different degrees. This suggests that ferulic and *p*-hydroxybenzoic acids may have inhibited some specific *Fusarium* species but not others. Fuchs et al. (1997) found that although *Fusarium* contain plant pathogens, most of *Fusarium* are saprophytic and some can induce systemic resistance in plants to protect them. Previous research studies have been demonstrated that exogenous ferulic and *p*-hydroxybenzoic acids stimulate the growth of cucumber wilt pathogens, which inhibits the growth of cucumber seedlings and aggravates soil disease (Jin et al. 2020). Thus, ferulic and *p*-hydroxybenzoic acids may have inhibited the beneficial *Fusarium* population of the plant without inhibiting the pathogenic strains. This study gives us further insight into the phenomenon of soil-borne diseases, pointing out that phenolic compounds may have different effects on specific soil microorganisms.

Conclusion

Results showed that exogenous ferulic and *p*-hydroxybenzoic acids had different effects on *Fusarium* community. High concentration of ferulic acid changed the community structure of *Fusarium*. All concentrations of *p*-hydroxybenzoic acid changed the community structure of *Fusarium*. All concentrations of ferulic and *p*-hydroxybenzoic acids significantly increased the community abundance of *Fusarium* in cucumber rhizosphere. Moreover, the abundance of *Fusarium* increased with the increase of ferulic and *p*-hydroxybenzoic acids concentration.

Competing Interests

No potential conflict of interest was reported by the author(s).

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References

Ahn, P., Chung, H., and Lee, Y. 1998. Vegetative compatibility groups and pathogenicity among isolates of *Fusarium oxysporum* f. sp. *cucumerinum*. Plant Disease, **82**(2): 244–246. doi:10.1094/pdis.1998.82.2.244. PMID: 30856809.

Armstrong, G.M., and Armstrong, J.K. 1981. Formae speciales and races of *Fusarium oxysporum* causing wilt disease. Fusarium: disease, biology, and taxonomy. Pennsylvania State University Press, University Park and London. 391–399. doi:10.1094/phyto-68-19.

Badri, D.V., Chaparro, J.M., Zhang, R., Shen, Q., and Vivanco, J.M. 2013. Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. J. Biol. Chem. **288**(7): 4502–4512. doi:10.1074/jbc.a112.433300.

Bardgett, R.D., and Van Der Putten, W. H. 2014. Belowground biodiversity and ecosystem functioning. Nature, **515**(7528): 505–511. doi:10.1038/nature13855. PMID: 25428498.

Bever, J.D., Platt, T.G., and Morton, E.R. 2012. Microbial population and community dynamics on plant roots and their feedbacks on plant communities. Annu. Rev. Microbiol. **66**(1): 265–283. doi:10.1146/annurev-micro-092611-150107.

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Booth, C. 1972. The Genus Fusarium. Taxon, **21**(1): 180–181. doi:10.2307/1219251.

Chen, S., Yu, H., Zhou, X., and Wu, F. 2018. Cucumber (*Cucumis sativus* L.) seedling rhizosphere *Trichoderma* and *Fusarium* spp. communities altered by vanillic acid. Front. Microbiol. **9**: 2195. doi:10.3389/fmicb.2018.02195. PMID: 30283420.

Fuchs, J.G., Moënne-Loccoz, Y., and Défago, G. 1997. Nonpathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt in tomato. Plant Disease, **81**(5): 492–496. doi:10.1094/PDIS.1997.81.5.492.

Gordon, T.R., and Martyn, R.D. 1997. The evolutionary biology of *Fusarium oxysporum*. Annu. Rev. Phytopathol. **35**(1): 111–128. doi:10.1146/annurev.phyto.35.1.111.

Huang, L.F., Song, L.X., Xia, X J., Mao, W.H., Shi, K., Zhou, Y.H., and Yu, J.Q. 2013. Plant-soil feedbacks and soil sickness: from mechanisms to application in agriculture. J. Chem. Ecol. **39**(2): 232–242. doi:10.1007/s10886-013-0244-9. PMID: 23385367.

Inderjit,Wardle, D.A., Karban, R., and Callaway, R.M. 2011. The ecosystem and evolutionary contexts of allelopathy. Trends Ecol. Evolut. **26**(12): 655–662. doi:10.1016/j.tree.2011.08.003.

Jin, X., Wu, F., and Zhou, X. 2020. Different toxic effects of ferulic and *p*-hydroxybenzoic acids on cucumber seedling growth were related to their different influences on rhizosphere microbial composition. Biol. Fertil. Soils **56**(1): 125–136. doi:10.1007/s00374-019-01408-0.

Lee, J.G., Lee, B.Y., and Lee, H.J. 2006. Accumulation of phytotoxic organic acids in reused nutrient solution during hydroponic cultivation of lettuce (*Lactuca sativa* L.). Sci. Horticulturae **110**(2): 119–128. doi:10.1016/j.scienta.2006.06.013.

Liu, J., Li, X., Jia, Z., Zhang, T., and Wang, X. 2016. Effect of benzoic acid on soil microbial communities associated with soilborne peanut diseases. Appl. Soil Ecol. **110**: 34–42. doi:10.1016/j.apsoil.2016.11.001.

Matsuyama, T., Nakajima, Y., Matsuya, K., Ikenaga, M., Asakawa, S., and Kimura, M. 2007. Bacterial community in plant residues in a Japanese paddy field estimated by RFLP and DGGE analyses. Soil Biol. Biochem. **39**(2): 463–472. doi:10.1016/j.soilbio.2006.08.016.

McKeen, C.D., and Wensley, R.N., 1961. Longevity of *Fusarium oxysporum* in soil tube culture. Science. **134**(3489): 1528–1529. doi:10.1126/science.134.3489.1528. PMID: 17800128.

Muzell Trezzi, M., Vidal, R.A., Balbinot Junior, A., von Hertwig Bittencourt, H., and da Silva Souza Filho, A.P. 2016. Allelopathy: driving mechanisms governing its activity in agriculture. J. Plant Interact. **11**(1): 53–60. doi:10.1080/17429145.2016.1159342.

Nelson, P. E., and Horts, R. 1983. Fusarium: diseases, biology and taxonomy. Mycologia, **75**(1): 190. doi:10.2307/3792947.

O'Donnell, K., Kistler, H.C., Cigelnik, E., and Ploetz, R.C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. **95**(5): 2044–2049. doi:10.1073/pnas.95.5.2044.

Pavlovkin, J., Mistrik, I., and Prokop, M. 2004. Some aspects of the phytotoxic action of fusaric acid on primary Ricinus roots. Plant Soil Environ. **50**(9): 397–401. doi:10.17221/4050-PSE.

Qu, X.H., and Wang, J.G. 2008. Effect of amendments with different phenolic acids on soil microbial biomass, activity, and community diversity. Appl. Soil Ecol. **39**(2): 172–179. doi:10.1016/j.apsoil.2007.12.007.

Ran, L., Li, J., Xing, Y., Zhang, J., and Zhou, X. 2021. Effects of p-Coumaric acid on the structure and abundance of soil *Pseudomonas* spp. community. Allelopathy J. **53**(2): 211–218. doi:10.26651/allelo.j/2021-53-2-1338.

Shafer, S.R., and Blum, U. 1991. Influence of Phenolic acids on microbial populations in the rhizosphere of cucumber. J. Chem. Ecol. **17**(2): 369–389. doi:10.1007/bf00994339. PMID: 24258732.

Sun, J.M., Fu, J.F., Zhou, R.J., and Yan, X.R. 2013. Antibiotic effects of four exogenous phenolic acids on soilborne pathogen, *Cylindrocarpon destructans*. In Applied Mechanics and Materials. Trans Tech Publications Ltd. Vol. **295-298**: 2294–2299. doi:10.4028/www.scientific.net/AMM.295-298.2294.

Van Der Heijden, M. G., Bardgett, R. D., and Van Straalen, N. M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. **11**(3): 296–310. doi:10.1111/j.1461-0248.2007.01139.x. PMID: 18047587.

Wakelin, S.A., Warren, R.A., Kong, L., and Harvey, P.R., 2008. Management factors affecting size and structure of soil Fusarium communities under irrigated maize in Australia. Appl. Soil Ecol. **39**(2): 201–209. doi:10.1016/j.apsoil.2007.12.009.

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

Yergeau, E., Filion, M., Vujanovic, V., and St-Arnaud, M. 2005. A PCR-denaturing gradient gel electrophoresis approach to assess Fusarium diversity in asparagus. J. Microbiolog. Methods **60**(2): 143–154. doi:10.1016/j.mimet.2004.09.006. PMID: 15590089.

Yu, J.Q., Shou, S.Y., Qian, Y.R., Zhu, Z.J., and Hu, W.H. 2000. Autotoxic potential of cucurbit crops. Plant Soil. **223**(1): 149–153. doi:10.1023/A:1004829512147.

Yu, J.Q., Ye, S.F., Zhang, M.F., and Hu, W.H. 2003. Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals, on photosynthesis and antioxidant enzymes in cucumber. Biochem. Systemat. Ecol. **31**(2): 129–139. doi:10.1016/s0305-1978(02)00150-3.

Zhang, J.H., Pan, D.D., Ge, X., Shen, Y.H., Qiao, P.L., Wu, F. Z., and Zhou, X.G. 2018. Effects of syringic acid on Fusarium and Trichoderma communities in cucumber (*Cucumis sativus* L.) seedling rhizosphere. Allelopathy J. **44**(2): 181–190. doi:10.26651/allelo.j./2018-44-2-1163.

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

Zhou, X., Liu, J., and Wu, F. 2017. Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth. Plant Soil **415**(1): 507–520. doi:10.1007/s11104-017-3181-5.

Zhou, X., Jia, H., Ge, X., and Wu, F. 2018. Effects of vanillin on the community structures and abundances of *Fusarium* and *Trichoderma* spp. in cucumber seedling rhizosphere. J. Plant Interact. **13**(1): 45–50. doi:10.1080/17429145.2017.1414322.

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