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Research Article

P-hydroxybenzoic acid positively affect the *Fusarium oxysporum* to stimulate root rot in *Panax notoginseng*

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A R T I C L E I N F O

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A B S T R A C T

Background: Plant health is directly related to the change in native microbial diversity and changes in soil health have been implicated as one of the main cause of root rot. However, scarce information is present regarding allelopathic relationship of *Panax notoginseng* root exudates and pathogenic fungi *Fusarium oxysporum* in a continuous cropping system.
Methods: We analyzed *P. notoginseng* root exudate in the planting soil for three successive years to determine phenolic acid concentration using GC-MS and HPLC followed by effect on the microbial community assembly. Antioxidant enzymes were checked in the roots to confirm possible resistance in *P. notoginseng*.
Results: Total 29 allelochemicals in the planting soil extract was found with highest concentration (10.54 %) of *p*-hydroxybenzoic acid. The HPLC showing a year-by-year decrease in *p*-hydroxybenzoic acid content in soil of different planting years, and an increase in population of *F. oxysporum*. Moreover, community analysis displayed negative correlation with 2.22 mmol. L^{-1} of *p*-hydroxybenzoic acid correspond to an 18.1 % population of *F. oxysporum*. Furthermore, *in vitro* plate assay indicates that medium dose of *p*-hydroxybenzoic acid (2.5–5 mmol. L^{-1}) can stimulate the growth of *F. oxysporum* colonies and the production of macroconidia, as well as cell wall-degrading enzymes. We found that 2–3 mmol. L^{-1} of *p*-hydroxybenzoic acid significantly increased the population of *F. oxysporum*.
Conclusion: In conclusion, our study suggested that *p*-hydroxybenzoic acid have negative effect on the root system and modified the rhizosphere microbiome so that the host plant became more susceptible to root rot disease.

1. Introduction

Fusarium oxysporum is one of the most prevalent phytopathogen results in crop losses. In planta, it induces *Fusarium* root rot with huge economic impact in many crop-growing areas around the world. The pathogen causes root rot and severely restricts the yield of strawberry, watermelon, cucumber production, and several others [1–3]. In most cases, poor crop growth and disease may be attributed to allelopathy, especially in *Panax notoginseng* [4]. Deciphering this allelopathy mechanism which is associated with the plants and pathogens, and the root exudates will advance to alleviate root rot disease in agricultural systems.

Allelopathy is release of bioactive metabolites into the surrounding environment such as root exudates are released into the rhizosphere which effectively influence interactions among neighboring plants and microbes [5]. Root exudation includes the sugars, amino acids, and complex secondary compounds. Phenolic acids are important plant secondary metabolites, which play important roles in plant-borne pathogens and plant-plant interactions [6]. Studies have shown that cinamic acid is related to Fusarium wilt infections and can significantly affect plant physiology and biochemistry [7,8]. Some documents reported, phenolic acids can also cooperate with pathogens to further increase infection rate and cause plant disease. The syringic acid is considered as a kind of allelochemical inducer, which is known to stimulate the relative virulence factors of invading pathogens [9]. P-coumaric acid was reported to change the composition of microbial

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communities in a cucumber rhizosphere and exerted negative effects on cucumber growth [6]. These results indicated that the phenolic acids have allelopathic effects on soil microbial communities and different compositions of soil microbial communities can provide different ecosystem functions. Understanding the relationships between phenolic acids and soil microbes may lead to devise solutions for plant disease control.

As a famous and precious herbal medicine, *P. notoginseng* has been used for more than one thousand years and the safety and efficacy of these medicines have been proven. The root rot disease of *P. notoginseng* is caused by *F. oxysporum*, a causal agent of Fusarium wilt or root rot disease in many crops such as peanuts, bananas, watermelon, and cucumbers [2,10–12]. *F. oxysporum* was reported to be the main pathogenic fungi, which is very difficult to remove from soil once it has been introduced [13]. Many studies have shown that cinamic acid can increase the risk of wilt disease by altering its physiological, biochemical, and defense responses of plants [6,14,15].

We hypothesized that *p*-hydroxybenzoic acid could negatively affect the growth of *P. notoginseng* and change its rhizosphere microbial community. Therefore, we investigated if *p*-hydroxybenzoic acid changed *P. notoginseng* soil microbial communities through the whole 3-year growth cycle of its growth. In addition, we also estimated the biomass of *F. oxysporum* and hydrolase resistance enzymes.

2. Materials and methods

2.1. Plant and fungal material

P. notoginseng seedlings were planted individually in plastic cups containing 150 g soil and incubated in a greenhouse at 25 °C. The soil was composed of red soil and sandy loam, which contained 3.67 % organic matter; 87.12 mg kg^{-1} available nitrogen; 65.26 mg kg^{-1} available phosphorus; and 108.35 mg kg^{-1} available potassium. The pathogenic strain is *F. oxysporum* E.F.Sm.& Swingle, which was preserved in Yunnan Key Laboratory of Authentic Chinese Medicinal Materials, at Yuxi Normal University, China.

2.2. GC-MS analysis of the allelochemical composition of soil cultivated with Panax notoginseng

Soil (500 g) was extracted with 500 mL of ethyl acetate and repeated 3 times. The fluid extracts were concentrated by rotary evaporator. The concentrated extracts in ethyl acetate were analyzed by GC-MS to identify the substance composition following the method described by Porter et al. [15]. The solution was filtered and diluted again twice, then performed GC-MS analysis. A fused silica column DB-5(length 30 cm; diameter 0.25 mm; thickness 0.25 μm) was equipped with GC-MS system. The extract (1 μL) was injected. The oven programming was carried out according to Porter et al. The initial oven temperature was 50 °C and held for 2 min, ramp 6 °C min^{-1} to 250 °C and held for 15 min. The final temperature was 280 °C and kept for 3 min. The ionization energy was 70 eV.

2.3. Determining the content of p-hydroxybenzoic acid in soil

2.3.1. Soil sample material

The soil samples were collected from Yanshan county, which is located in the Eastern part of Wenshan Zhuang Autonomous Prefecture (23°23′N, 103°30′E), Yunnan province, China. Rhizosphere soil of *P. notoginseng* were collected in plots corresponding with planting years of 1 year, 2 years, and 3 years using a five-spot-sampling method. Soil without *P. notoginseng* plantings was used as control.

2.3.2. Determining the content of p-hydroxybenzoic acid

Phenolic acids compounds were extracted using the method described by Refs. [16,17].

Determination of phenolic acid concentration was investigated using high performance liquid chromatography (HPLC) equipment (Agilent 1260 Infinity, USA) with a C18 column (4.6 mm × 250 mm). The sample (1 μL) was injected; the mobile phases consisted of solvents A (methanol) and solvents B (phosphoric acid solution with concentration 0.1 %). Elution was carried out with phosphoric acid solution and flow rates were: 0–15 min, phosphoric acid solution was reduced from 80 to 5 %, 15–18 min held conditions increased to 80 %, 18–20 min phosphoric acid solution drop to 0 % and stop to 25 min; flow rate 0.5 mL min^{-1}.

2.4. Analytical method and data analysis of soil microbial communities

2.4.1. 18S rRNA gene sequencing and analysis

DNA extraction method was conducted according to our previous literature description [18]. The ACE, Chao estimator, Shannon, and Simpson diversity were calculated to measure the alpha diversity analysis. The 18S rRNA gene was amplified with specific fungi primer NS1-FUNG. Raw sequences were analyzed described by Mason and Stolze [19,20].

2.5. Evaluation of root rot

The disease severity was based on the yellowing area of leaf. We divided the disease severity into four levels: the zero level has no yellowing symptoms; the first level leaf yellowing area is less than 25 %; the second level leaf yellowing area is from 26 % to 50 %; the third level leaf yellowing area is from 51 % to 75 % and plant wilting; the level 4 leaf yellowing area is more than 75 % and plant displays severe wilting. Incidence was calculated refer to the reported study [1]. Disease incidence was calculated by the proportion of diseased plants.

2.6. Measurement of biomass production and hydrolase activity

The mycelial growth was used to evaluate the growth of fungus, and potato dextrose agar (PDA) was used as growth media. The *F. oxysporum* was inoculated in 7 kinds of *p*-hydroxybenzoic acid concentration PDA medium (0, 1.5, 2.5, 5, 7.5, 10, and 50 mmol L^{-1} respectively), and incubated at 28 °C for 7 days.

The biomass production of cellulase and pectinase were analyzed according to the Wu et al. method [10,11,21,22].

2.7. Determining resistance enzyme in Panax notoginseng seedlings

The soil of planting contained different concentrations of *p*-hydroxybenzoic acid. The *p*-hydroxybenzoic acid added into each soil was rapidly depleted due to the microbial use under optimum environmental conditions [23]. The *p*-hydroxybenzoic acid was added to the planting soil every 7 days to maintain the desired levels.

Soil containing *P. notoginseng* seedlings was treated with *p*-hydroxybenzoic acid at concentrations of 0, 1, 2.5, 5, and 10 mmol L^{-1}, 5 times every 2 days. Total 10 seedlings were used as one treatment and replicated three times. The 0.1 M NaOH was used to adjust pH and soil moisture was maintained at 60 %.

Antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were analyzed according to the Wu et al. method [21].

2.8. Analysis method

The difference of date between analyzed were subjected to one-way or two-way ANOVA ($\alpha < 0.05$) in R-Studio. Through clustering operation, the sequence was divided into many groups according to their similarity, and a group represents an OTU. All sequences can be divided into OTUs according to different similarity levels. Usually, the OTUs at 97 % similarity level are analyzed by bioinformatics statistics. The S_{obs} is number of OTUs; the n_i is number of sequences contained in the OTU,

and the N is the number of all individuals, adapted here to be the total number of gene sequences.

3. Results

3.1. Characterization of the allelochemicals composition in soil

GC-MS analysis of the extractions identified more than 51 compounds. A comparison of mass spectra peaks and retention times with reference soil matrices allow us to reduce the results down to 29 significant compounds. These included organic acids, esters, hydrocarbons, phenols, and heterocyclic compounds. The main compounds were *p*-hydroxybenzoic acid, ethyl benzene, butyl propionate, benzoic acid, di-isobutyl phthalate, o-xylene, m-xylene, butyl acetate, with the proportions of 10.54, 9.32, 8.54, 6.21, 5.65, 5.08, 4.77, 3.11 %, respectively.

The chemicals identified in soil of cultivated *P. notoginseng* were subjected to further analysis to determine the major components. It was shown that the phenolic acids were the main compounds in soil of cultivated *P. notoginseng*. Of these, benzoic acid (6.21 %) and *p*-hydroxybenzoic acid (10.54 %) were shown to be the chief components (see Table 1).

3.2. Determining the content of p-hydroxybenzoic acid in soil

HPLC methods have been employed to determine the contents of *p*-hydroxybenzoic acid in rhizosphere soil samples of different *P. notoginseng* planting years. The results showed that the concentration of *p*-hydroxybenzoic acid in rhizosphere soil samples from the control, first years, second year and third year were 3.48 mmol L^{-1}, 5.11 mmol L^{-1}, 3.95 mmol L^{-1}, and 2.22 mmol L^{-1} respectively (Table 2). The maximum value of *p*-hydroxybenzoic acid was 5.11 mmol L^{-1}, taken from the soil that was planted with *P. notoginseng* for one year. This is 1.47 times higher than the control soil acid concentration. The *p*-hydroxybenzoic acid in the soil cultivated for two years was 3.95 mmol L^{-1}, which is 1.14 times higher than the control soil. The *p*-hydroxybenzoic acid concentration in soil cultivated for three years was 2.22 mmol L^{-1}, which was lower than the control soil. From the differences in *p*-hydroxybenzoic acid between the soil samples of each planting year and their corresponding control soil samples, we observed the soil with the highest *p*-hydroxybenzoic acid was the soil planted with *P. notoginseng* for one year. The content of *p*-hydroxybenzoic acid in the soil around the roots of *P. notoginseng* decreased over time. The concentration of *p*-hydroxybenzoic acid increased most significantly in the first year following planting.

Table 1
Allelochemicals in soil of cultivated *Panax notoginseng* identified by GC-MS.

Number	Retention time	Compound name	Area (%)
1	4.331	Butyl acetate	3.11
2	5.343	Ethyl benzene	9.32
3	5.529	O-xylene	5.085
4	6.100	M-xylene	4.768
5	8.825	1,3,5-trimethylbenzene	0.304
6	10.047	Butyl propionate	8.547
7	10.463	1,4-diethylbenzene	0.995
8	11.495	1,3-dimethyl-4-ethylbenzene	0.605
9	12.392	3,5-diethyltoluene	1.169
10	12.512	1,2,3,5-tetramethylbenzene	0.723
11	13.013	Acetylbenzene	2.921
12	13.203	P-hydroxybenzoic acid	10.54
13	13.273	Benzoic acid	6.21
14	13.409	P-ethyl toluene	0.293
15	14.145	N-undecanone	31.516
16	14.406	N-tridecane	0.534
17	14.441	N-Pentadecane	0.667
18	14.876	N-Heptadecane	1.287
19	15.107	N-octadecane	0.724
20	15.302	N-eicosane	0.365
21	15.979	N-octadecyl cyclohexane	0.279
22	19.896	Decamethylcyclopentasiloxane	4.431
23	21.205	Diisobutyl phthalate	5.65
24	21.865	Butyl phthalate 2-ethylhexyl ester	5.161
25	24.450	Pentadecane	0.171
26	24.996	Hexadecane	0.292
27	25.326	Octacosane	1.905
28	25.552	Methyl tetradecanoate	0.322
29	26.198	Triacontanol	0.580

Table 2
Content of *p*-hydroxybenzoic acid, density of *Fusarium oxysporum* in soils and incidence of root rot with different planting years of *Panax notoginseng*.

Soil sample	Content of *p*-hydroxybenzoic acid (mmol.L^{-1})	Population density of *F. oxysporum* (%)	Incidence of root rot (%)
CK	3.48	4.06 %	3.5 %
First year	5.11	1.07 %	3 %
Second year	3.95	7.1 %	9.3 %
Third year	2.22	18.1 %	16.7 %

3.3. Composition of fungal communities in Panax notoginseng rhizosphere from different planting year

Fungal communities were analyzed for mixed liquor suspended solids (MLSS), collected from the soil of different planting years. The microbial diversity indices of the normalized sample from each planting year and the composition of the microbial community indicated that community structures of each sample were significantly different. Total number of operational taxonomic units (OTUs) were significantly changed from first to third year (Table S1). We identified 11 fungi from the genus level analysis: *Epicoccum*, *Fomitopsis*, *Fusarium*, *Chaetomium*, *Verticillium*, *Cladosporium*, *Chondrostereum*, *Galerina*, *Penicillium*, *Cyathus,* and *Paecilomyces* (Fig. 1). The genus *Fomitopsis* was 32.48 % abundant and it was the most dominant genus identified in planting soil in the first year. In the second-year soil, the most dominant genus was *Epicoccum* and *Fusarium*, having 8.15 % and 7.1 % respectively. In the third-year soil, the genus *Fusarium* replaced the genus *Epicoccum* as the dominant genus, reaching 18.1 %. These results suggested that several species of *Fusarium* are responsible to cause root rot on *P. notoginseng*.

Considering *p*-hydroxybenzoic acid with the fungal community analysis, the results showed that *F. oxysporum* had the highest proportion (18.1 %) when the content of *p*-hydroxybenzoic acid was 2.22 mmol L^{-1} (Table 2). The relative abundances of *F. oxysporum* decreased with the increased concentration of *p*-hydroxybenzoic acid. *F. oxysporum* had 1.07 % abundance when the concentration of *p*-hydroxybenzoic acid was 5.11 mmol L^{-1} (Fig. 1). We found that high concentration of *p*-hydroxybenzoic acid inhibited the growth of *F. oxysporum*.

In addition, we have detected four pathogens related to the occurrence of *P. notoginseng* root rot in soil samples, namely *F. solani*, *Rhizoctonia solani*, *Cylindrocarpon destructans*, and *Clonostachys rosea*. Among these pathogens, the allelopathic effects of *p*-hydroxybenzoic acid on *C. destructans* and *F. solani* were similar to those on *F. oxysporum*. When the content of *p*-hydroxybenzoic acid was 2.22 mmol L^{-1}, the abundance of *C. destructans* and *F. solani* was the highest, with *C. destructans* being 0.07 % and *F. solani* being 0.18 %. When the content of *p*-hydroxybenzoic acid was 5.11 mmol L^{-1}, no *C. destructans* was detected, whereas the abundance of *F. solani* was 0.04 %. The allelopathic effects of *p*-hydroxybenzoic acid on *R. solani* were in contrast to *F. oxysporum*. When the content of *p*-hydroxybenzoic acid was 2.22 mmol L^{-1}, the abundance of *Rhodotonia solani* was the lowest at 0.02 %. When the content of *p*-hydroxybenzoic acid was 5.11 mmol L^{-1}, the abundance of *R. solani* was the highest at 0.69 %. The abundance of *Clonostachys rosea* was not correlated with the changes in *p*-

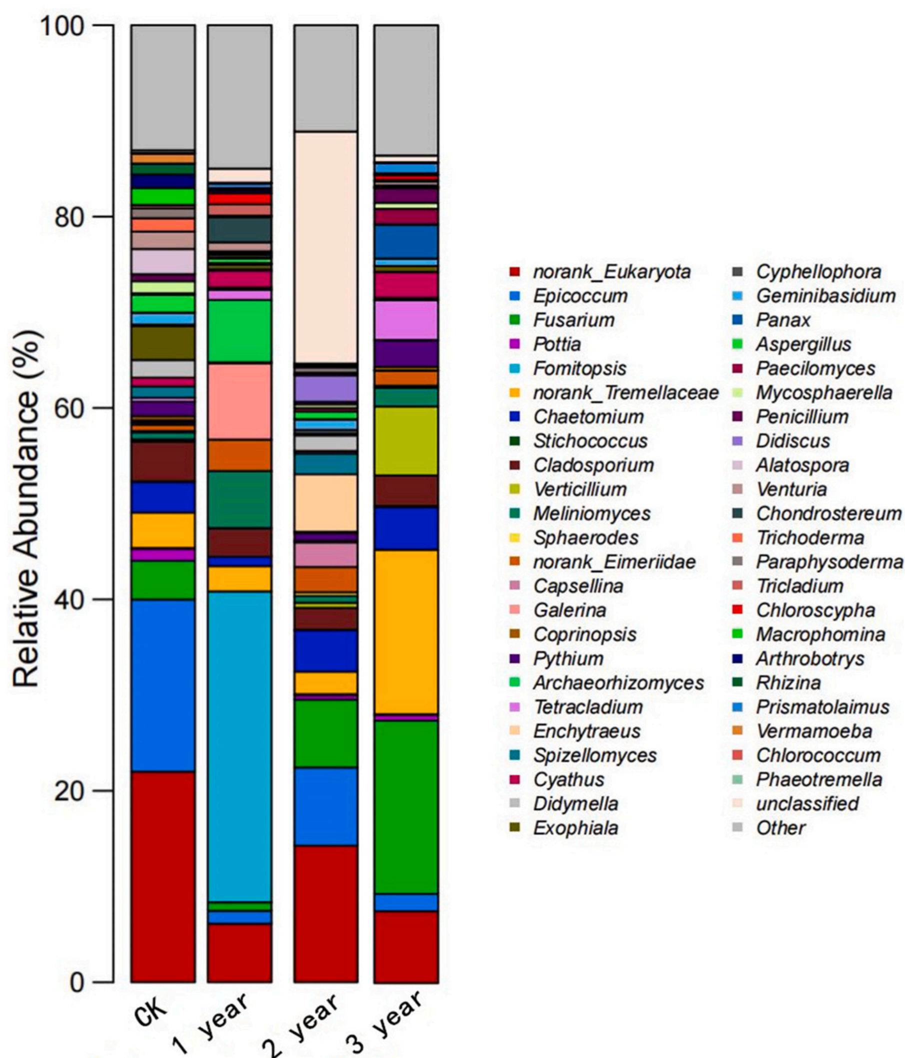

Fig. 1. Samples community structure distribution charts. The horizontal axis is the number of each sample, and the vertical axis is the relative abundance ratio of species. The color corresponds to the name of each species at this taxonomic level. Different color block widths indicate the relative abundance ratios of different species.

hydroxybenzoic acid content in the soil.

In the whole growth period of *P. notoginseng*, we found that the incidence and disease index of root rot in *P. notoginseng* plants were highest in the third year, followed by the second year, in that order (Table 2). In the third-year planting stages, *F. oxysporum* populations significantly increased the wilt incidence rate. Taken together, suitable concentration of *p*-hydroxybenzoic acid promoted the occurrence of root rot.

3.4. Measurement of biomass production under p-hydroxybenzoic acid stress

The biomass of *F. oxysporum* was analyzed in different *p*-hydroxybenzoic acid environment. To observe the *p*-hydroxybenzoic acid effect on *F. oxysporum* spores and mycelium, 7 culture dish treatments were used with different acid concentrations of 0, 1, 2.5, 7.5, 10, and 50 mmol L^{-1}. The results showed that different *p*-hydroxybenzoic acid concentrations resulted in different allelopathy. First, the mycelial growth of *F. oxysporum* was inhibited by *p*-hydroxybenzoic acid at concentrations ranging from 7.5 mmol L^{-1} to 50 mmol L^{-1}. In addition, the *F. oxysporum* had smaller colonies and the mycelial growth was dense. Secondly, in low acid concentration treatments between 2.5 and 5 mmol L^{-1}, the colony growth rate of *F. oxysporum* increased. The colony was larger compared with the control and the color was darker, but its phialide was denser, and microspores were observed by microscope. Thirdly, there was no significant difference in colony growth between treatments of less than 1 mmol L^{-1}, when compared with the control. Table 3 shows colony growth and spore production under the different *p*-hydroxybenzoic acid concentrations.

In addition, megaspores were observed when *p*-hydroxybenzoic acid content was 5 mmol L^{-1} by microscope. Some reports show that *F. oxysporum* generally produces microspores but do not produce megaspores on a PDA medium.

Table 3
Biomass of *Fusarium oxysporum* under the *p*-hydroxybenzoic acid stress.

Content of P-hydroxybenzoic acid (mmol L^{-1})	Colony diameter (mm)	Number of conidia (10^4.mL^{-1})
0	89 ± 0.6cd	130 ± 0.6b
1	89 ± 2.5cd	142 ± 1.0c
2.5	92 ± 1.2d	170 ± 0.8c
5	92 ± 0.6d	190 ± 1.2b
7.5	86 ± 0.4c	100 ± 1.0b
10	81 ± 1.1b	80 ± 9.5 b
50	40 ± 0.8a	0

Note: Different letters displayed significant difference ($p < 0.05$).

3.5. Pathogenic enzyme activity analysis

The *p*-hydroxybenzoic acid at concentrations ranging from 2.5 mmol L^{-1} to 10 mmol. L^{-1} has a dose-dependent relation to these enzymes. The pectinase enzyme was 1.02 U. ml^{-1}. min^{-1} in the presence of 2.5 mmol L^{-1} p-hydroxybenzoic acid. The production of the cellulase was stimulated by *p*-hydroxybenzoic acid. For example, in the presence of 50 mmol L^{-1} acid, the production of the pectinases was completely promoted and cellulases increased by 87 % (Fig. 2). However, at concentrations from 2.5 mmol L^{-1} to 10 mmol L^{-1}, *p*-hydroxybenzoic acid did not influence cellulase production.

3.6. Analysis of resistance enzyme in Panax notoginseng seedlings and root rot disease severity

The antioxidant root resistance enzymes of *P. notoginseng* seedlings changed significantly compared with the control (Fig. 3). In

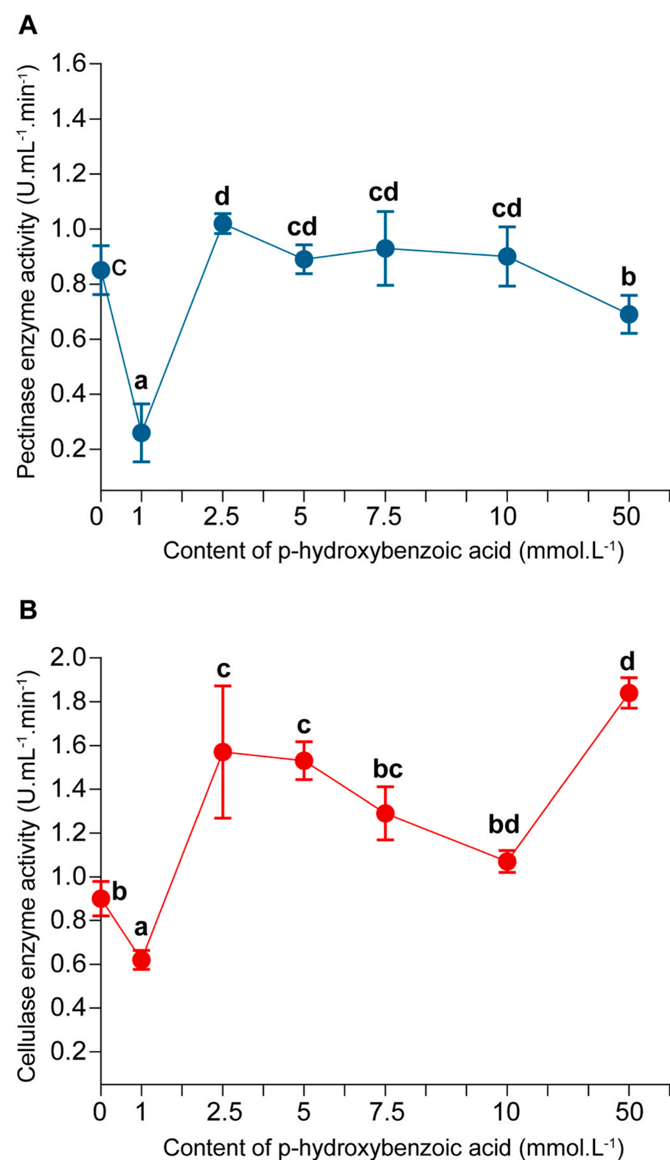

Fig. 2. Effects of *P*-hydroxybenzoic acid on pathogenic enzyme. Pectinase enzyme activity (expressed as U.ml^{-1}.min^{-1} protein), cellulase enzyme activity (expressed as U.ml^{-1}.min^{-1} protein) in mycelial of *F. oxysporum* on a time course after treated with *p*-hydroxybenzoic acid. Date presented in graphs are the means ± SD of three replicates. Different letters displayed significant difference ($p<0.05$).

P. notoginseng seedlings, activity of SOD was stimulated by 1–5 mmol L^{-1} of *p*-hydroxybenzoic acid, while 10 mmol L^{-1} of acid decreased SOD activity. A substantial increase of POD activity was observed with treatment by low concentrations of 0–5 mmol L^{-1} *p*-hydroxybenzoic acid. POD activity reached a peak in activity of 3.9 U g^{-1} at the acid concentration of 1 mmol L^{-1}. This suggests that high concentrations of *p*-hydroxybenzoic acid had a detrimental effect on the root system of *P. notoginseng*. The highest activity of CAT was 0.235 U g^{-1}, which was obtained at 2.5 mmol L^{-1} concentration of *p*-hydroxybenzoic acid. CAT activity was reduced at higher concentration of *p*-hydroxybenzoic acid (5–10 mmol L^{-1}).

We noted that the *P. notoginseng* began to develop symptoms of root rot gradually after 30 days when treated with *p*-hydroxybenzoic acid, while no disease severity was observed in *P. notoginseng* treated with 10 mmol L^{-1} *p*-hydroxybenzoic acid and distilled water. During the treatment period, the *P. notoginseng* treated with 5 mmol L^{-1} *p*-hydroxybenzoic acid demonstrated the highest disease severity, which was increased by 13.5 % as compared to control. The disease severity was 3.6 % and 6.8 % with 1 mmol L^{-1} and 2.5 mmol L^{-1} *p*-hydroxybenzoic acid respectively.

4. Discussion

4.1. Variation of P-hydroxybenzoic acid content in soil during a planting period of Panax notoginseng

Phenolic acid compounds are a main factor influencing continuous cropping methods, which affect crop growth by promoting or inhibiting microbial communities, directly or indirectly [1]. *P*-hydroxybenzoic acid is a common phenolic compound produced by plants, and exists in higher plants, microorganisms, moss, and soil [24]. In this study, the components of allelochemicals in *P. notoginseng* planting soil were analyzed by GC-MS and we focused on the phenolic compound *p*-hydroxybenzoic acid because of its high content in the soil. This study tracked and analyzed the changes of soil *p*-hydroxybenzoic acid content and the response of microbial community structure during the 3-year planting period of *P. notoginseng.* At the same time, with the decrease of *p*-hydroxybenzoic acid content, the population density of *F. oxysporum* increased, and there was an inverse relationship between them. Related research shows that *p*-hydroxybenzoic acid can stimulate soil respiration, microbial biomass, and amylase activity when it enters the soil with tillage, and it can be used as a carbon source that is degraded in the soil by some microorganisms [25]. In this study, the content of *p*-hydroxybenzoic acid decreased, which may be because it is a carbon source of *F. oxysporum* and promotes its growth. Therefore, the content of *p*-hydroxybenzoic acid in soil is closely related to the population density of *F. oxysporum*.

The growth cycle of *P. notoginseng* is generally three to four years, and, currently, the artificial planting cycle is generally three years. In this study, we monitored the content of *p*-hydroxybenzoic acid in the rhizosphere soil and changes in rhizosphere microorganisms in the first, second, and third years of *P. notoginseng* cultivation. However, for plants of the Panax genus, the planting time is generally three to seven years or longer. In future, the experiment will be improved by extending the planting time of *P. notoginseng* to obtain more comprehensive data and to further explain the relationship between changes in *p*-hydroxybenzoic acid content and the rhizosphere microorganism community structure. Salicylic acid, is an allelopathic substance, could stimulate the production of mycotoxins and the activity of hydrolase in *F. oxysporum* but inhibits its growth, conidia formation, and germination to some extent [26]. Thymol (0.19 g·L-1) and salicylic acid (0.16 g·L-1) could severely affect the cell structure and morphology of *F. solani* by disrupting the integrity of the cell membrane, with more adverse effects with salicylic acid than with thymol [27]. Przybylska-Balcerek (2022) evaluated the antimicrobial effects of phenolic acid extracts from natural infection and microorganism inoculation. The results showed that 17 extracts had

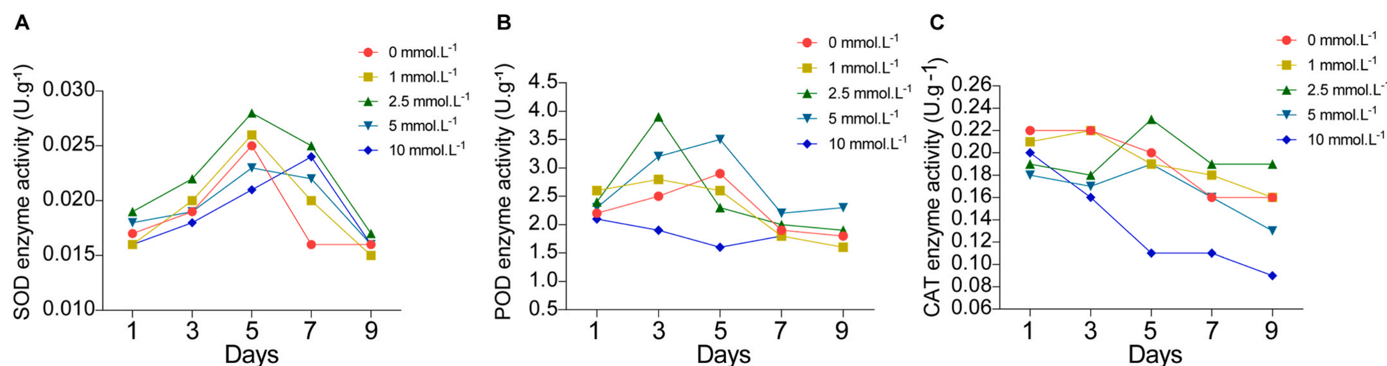

Fig. 3. Effects of *P*-hydroxybenzoic acid on resistance enzymes. Superoxide dismutase activity (expressed as $U.g^{-1}$ protein), catalase activity (expressed as $U.g^{-1}$ protein) and peroxidase activity (expressed as $U.g^{-1}$ protein) in roots of *P. notoginseng* seedlings on a time course after treated with *p*-hydroxybenzoic acid. Date presented in graphs are the means ± SD of three replicates. Different letters displayed significant difference ($p<0.05$).

fungicidal effects on *F. culmorum* (FC) (NIV), 16 extracts had fungicidal effects on *F. culmorum* (FC) (3AcDON), 12 extracts were fungicidal towards *F. graminearum* (FG) (3AcDON), 12 extracts had fungicidal effects on *F. graminearum* (FG) (NIV), and 19 extracts had fungicidal effects on *F. langsethiae* (FL) [28].

4.2. Effect of p-hydroxybenzoic acid on pathogenicity of Fusarium oxysporum

In this study, 2.5–5 mmol L^{-1} *p*-hydroxybenzoic acid had the strongest allelopathy and promoted *F. oxysporum* growth under culture dish experiments conditions. In this concentration range of *p*-hydroxybenzoic acid, the colony diameter and the microspore production of *F. oxysporum* increased and promoted the production of megaspores. Studies have shown that the allelopathic effect of phenolic acids on pathogenic fungi can induce proliferation, and promote the secretion of secondary metabolites, including toxins and cell wall degrading enzymes [29]. For example, *F. oxysporum* contains β- 1,3 glucanase, pectinase, and cellulase which can affect their pathogenicity, and it also contains genetic code for endo-polygalacturonase, exo-galacturonidase, pectinate endolyase, and xylanase. Knocking out any gene for these proteins will lead to the complete or partial loss of pathogenicity of *F. oxysporum* to the host. In this study, medium concentrations (2.5–10 mmol L^{-1}) of *p*-hydroxybenzoic acid stimulated the activity of pectinase [30]. The activity of cellulase was stronger under the high 50 mmol L^{-1} acid concentration. This may be because the secretion of cell wall degrading enzymes such as pectinase and cellulase is affected by microbial carbon and nitrogen sources [31,32]. The addition of *p*-hydroxybenzoic acid in the culture medium increases the available carbon source and promotes the secretion of pectinase and cellulase.

5. Conclusions

Based on overall study, our results showed that *p*-hydroxybenzoic acid, a phenolic acid compound that is detected in rhizosphere soil, altered the native soil microbial communities and increased the amount of *F. oxysporum* in the rhizosphere. In addition, it also changed the rhizosphere soil enzyme activities, and reduced the resistance of *P. notoginseng*. These factors have negative effect in one way or another to inhibit *P. notoginseng* growth and promote Fusarium root rot disease, however, more detailed mechanisms need to be studied in the future. In summary, our results provided the relationship between the abundance of *F. oxysporum* and the content of *p*-hydroxybenzoic acid. Finally, we concluded that *p*-hydroxybenzoic acid is an important factor causing increased root rot in *P. notoginseng* further increase the secretion of pathogenic enzymes.

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Contributions

Z.J. conceived the idea. Z.J., W,Z., J.R. designed and carried out the experiments. S.M., W.Q and W.L prepared figures. L.L and Y.Y. performed data analysis. S.M and Z.J. drafted the final version of the manuscript.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2023.11.005.

References

[1] Tian GL, Bi YM, Cheng JD, Zhang FF, Zhou TH, Sun JZ, Zhang LS. High concentration of ferulic acid in rhizosphere soil accounts for the occurrence of fusarium wilt during the seedling stages of strawberry plants. Physiol Mol Plant Pathol 2019;108:101435–55. https://doi.org/10.1016/j.pmpp.2019.101435.
[2] Ma K, Kou J, Rahman M, Du W, Pan K. Palmitic acid mediated change of rhizosphere and alleviation of fusarium wilt disease in watermelon. Saudi J Biol Sci 2021;(2):512–26. https://doi.org/10.1016/j.sjbs.2021.03.040.
[3] Gao H, Shi YJ, Jin X, Wang J, Wu FZ, Zhou XG. Effects of p-hydroxybenzoic acid on cucumber rhizosphere Trichoderma spp. community structure and abundance. Allelopathy J 2019;47(1):93–102. https://doi.org/10.26651/allelo.j/2019-47-1-1222.
[4] Mao ZS, Long YJ, Zhu YY, Zhu SS, He XH, Chen ZJ. First report of Cylindrocarpon destructans var. destructans causing black root rot of sanqi (Panax notoginseng) in China. Plant Dis 2014;98:162. https://doi.org/10.1094/PDIS-11-12-1104-PDN.
[5] Zhang DQ, Yan DD, Cheng HY, Fang WS, Huang B, Wang XL, Wang XN, Yang y, Ouyang CB, Wang QX, Cao oc. Effects of multi-year biofumigation on soil bacterial and fungal communities and strawberry yield. Environ. Pollut. 2020;256. 10.10.1016/j.envpol.2019.113415.
[6] Zhou X, Zhang J, Pan D, Ge X, Jin X, Chen S, Wu F. p-Coumaric can alter the composition of cucumber rhizosphere microbial communities and induce negative plant-microbial interactions. Biol Fertil Soils 2018;54:363–72. https://doi.org/10.1007/s00374-018-1286-5.

[7] Huang LF, Song LX, Xia J, Mao WH, Shi K, Zhou YH. Plant-soil feedbacks and soil sickness: from mechanism to application in agriculture. J Chem Ecol 2013;39(2):232–42. https://doi.org/10.1007/s10886-013-0244-9.
[8] Inderjit Wardle DA, Karban R, Callaway RM. The ecosystem and evolutionary contexts of allelopathy. Trends Ecol Evol 2011;26(12):655–62. https://doi.org/10.1016/j.tree.2011.08.003.
[9] Wu HS, Luo J, Liu YX, Chen AQ, Tang Z, Chao Y, Chen G, Mao ZS, Huang W, Shen QR. In vitro physiological responses of Fusarium oxysporum f. sp. niveum to exogenously applied syringic acid. J Eukaryot Microbiol 2009;56(4):386–7. https://doi.org/10.1111/j.1550-7408.2009.00417.x.
[10] Wu HS, Yin XM, Liu DY, Ling N, Bao W, Ying RR, Zhu YY, Guo SW, Shen QR. Effect of fungal fusaric acid on the root and leaf physiology of watermelon (Citrullus lanatus) seedlings. Plant Soil 2008;308:255–66. https://doi.org/10.1007/s11104-008-9627-z.
[11] Wu HS, Waseem R, Fan JQ, Sun YG, Bao W, Liu DY, Ling N, Mao ZS, Shen QR, Miao WG. Antibiotic effect of exogenously applied sal icylic acid on in vitro soilborne pathogen. Fusarium oxysporum f. sp. niveum. Chemosphere 2008;74(1):45–50. https://doi.org/10.1016/j.chemosphere.2008.09.027.
[12] Jin X, Wu F, Zhou X. 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 2020;56:642–50. https://doi.org/10.1007/s00374-019-01408-0.
[13] Wang GC, Chen HK, Luo PX. Isolation, inoculation and fungicidal tests on the pathogens causing root rot of Panax *Panax notoginseng* Wall. var. Notoginseng (Burkill) Hoo & Tseng. Acta Phytopathol Sin 1991;21(2):66.
[14] Miao CP, Mi QL, Qiao XG, Zheng YK, Zhao LX. Rhizospheric fungi of *panax notoginseng*: diversity and antagonism to host phytopathogens. Journal of Ginseng Research 2016;40(2):127–34. https://doi.org/10.1016/j.jgr.2015.06.004.
[15] Porter PM, Banwart WL, Hassett JJ. HPLC and GC-MS identificaton of genistein, daidzein and coumestrol from unhydrolyzed sobean root extracts. Environ Exp Bot 1985;25:29–232. https://doi.org/10.1016/0098-8472(85)90006-1.
[16] Pagnussatt FA, Delponte EM, Garda-Buffon J, Badiale-Furlong E. Inhibition of fusarium graminearum growth and mycotoxin production by phenolic extract from spirulina sp. Pestic Biochem Physiol 2014;108:21–6.
[17] Scaglioni PT, Blandino M, Scarpino V, Giordano D, Testa G, Badiale-Furlong E. Application of fungicides and microalgal phenolic extracts for the direct control of fumonisin contamination in maize. J Agric Food Chem 2018;66(19):4835–41. https://doi.org/10.1021/acs.jafc.8b00540.
[18] Zhao J, Li M, Liu Q, Zhou ZM, Ye CB. Addition of chitosan improves the effificiency of total phosphorus removal from wastewater using the D-A2O reactor and metagenomic analysis. Water Pol 2021;23(6):1530–41. https://doi.org/10.2166/wp.2021.065. 2021.
[19] Mason OU, Scott NM, Gonzalez A, Robbins-Pianka A, Bælum J, Kimbrel J, Bouskill NJ, Prestat E, Borglin S, Joyner DC, Fortney JL, Jurelevicius D, Stringfellow WT, Alvarez-Cohen L, Hazen TC, Knight R, Gilbert GA, Jansson JK. Metagenomics reveals sediment microbial community response to deepwater horizon oil spill. ISME J 2014;8(7):1464–75. https://doi.org/10.1038/ismej.2013.254.
[20] Stolze Y, Zakrzewski M, Maus I, Eikmeyer F, Jaenicke S, Rottmann N, Siebner C, Puhler A, Schluter A. Comparative metagenomics of biogas-producing microbial communities from production-scale biogas plants operating under wet or dry fermentation conditions. Biotechnol Biofuels 2015;8(1):14. https://doi.org/10.1186/s13068-014-0193-8.
[21] Wu HS, Waseem R, Liu DY, Wu CL, Mao ZS, Shen QR. Allelopathic impact of artifificially applied coumarin on Fusarium oxysporum f. sp. niveum. World Journal of Microbiology and Biochemistry 2008;24:1297–304. https://doi.org/10.1007/s11274-007-9602-5.
[22] Wu HS, Waseem R, Fan JQ, Sun YG, Bao W, Shen QR. Cinnamic acid inhibits growth but stimulates production of pathogenesis factors by in vitro cultures of Fusarium oxysporum f. sp. niveum. J Agric Food Chem 2008;56:1316–21. https://doi.org/10.1021/jf0726482.
[23] Shafer SR, Blum U. Influence of phenolic acids on microbial populations in the rhizosphere of cucumber. J Chem Ecol 1991;17:369–89. https://doi.org/10.1007/BF00994339.
[24] Lv JX, Li L, Chen L, Guo YT, Dong K, Dong Y. Salicylic acid has allelopathy, but wheat and faba bean intercropping can alleviate faba bean fusarium wilt under salicylic acid stress. Research Square 2021;22(5):132–54. https://doi.org/10.21203/rs.3.rs-228506/v1.
[25] Emad K, Sujitraj S, Wei W, Wang XG. Determination of phenolic compounds in gurum(*Citrulluslanatus var. Colocynthoide*) seed oil obtained by different methods using HPLC. Food analitical methods 2020;13(5):264–78. https://doi.org/10.1007/s12161-020-01757-9.
[26] Kong J, Xie YF, Yu H, Guo YH, Cheng YL, Qian H, Yao WR. Synergistic antifungal mechanism of thymol and salicylic acid on. Fusarium solani 2021;140(1):81–9.
[27] Wu HS, Raza W, Fan JQ, Sun YG, Bao W, Liu DY, Huang QW, Mao ZS, Shen QR, Miao WG. Antibiotic effect of exogenously applied salicylic acid on in vitro soilbotne pathogen. Fusarium oxysporum f.sp.niveum. 2008;74:45–50.
[28] Przybylska-Balcerek A, Szablewski T, Cegielska-Radziejewska R, Góral T, Kurasiak-Popowska D, Stuper-Szablewska K. Assessment of antimicrobial properties of phenolic acid extracts from grain infected with fungi from the genus. Fusarium 2022;27(5):1741–62.
[29] Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA 2006;103:626–31. https://doi.org/10.1073/pnas.0507535103.
[30] Jonkers W, Rodrigues CD, Rep M. Impaired colonization and infection of tomato roots by the deltafrp1 mutant of Fusarium oxysporum correlates with reduced CWDE gene expression. Molecular plant-microbe interactions 2009;22(5):507–18. https://doi.org/10.1094/MPMI-22-5-0507.
[31] Zwetsloot MJ, Kessler A, Bauerle TL. Phenolic root exudate and tissue compounds vary widely among temperate forest tree species and have contrasting effects on soil microbial respiration. New Phytol 2018;2(21):156–78. https://doi.org/10.1111/nph.15041.
[32] Schöneberg T, Kibler K, Sulyok M, Musa T, Bucheli TD, Mascher F, Bertossa M, Voegele RT, Vogelgsang S. Can plant phenolic compounds reduce fusarium growth and mycotoxin production in cereals? Food Addit Contam 2018:1–16. https://doi.org/10.1080/19440049.2018.1538570.