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Original Research

Root Exudates of *Wedelia trilobata* Suppress Soil-Borne Pathogenic Fungi and Increase Its Invasion

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Abstract

Invasive species have competitive advantages over neighboring native species and decrease the biodiversity of the local community. The novel weapon hypothesis suggests that invasive plants affect local plants and suppress soil-borne pathogens by exuding allelochemicals to facilitate invasion. Therefore, the following study was designed by focusing on the extracts derived from certain parts of plants, and the impact of the collected root exudates of invasive plants on soil-borne pathogens has been documented, but there is still a need to identify its mechanisms. We conducted several experiments to test whether the alien invasive plant species *Wedelia trilobata* (L.) Hitchc. can suppress soil-borne pathogens through its root exudates. We also attempted to explain the inhibition mechanism of the root exudates on soil-borne pathogens. The results showed that the root exudates of *W. trilobata* significantly inhibited the growth and activities of soil-borne pathogens under sterile petri dish and sand cultures. Specifically, root exudates decreased the conidial germination, mycotoxin production, and hydrolytic enzyme (pectinase, cellulase, and amylase) activities of soil-borne pathogens in liquid culture, indicating the inhibition mechanism of root exudates of *W. trilobata* on soil-borne pathogens. Our results provided direct evidence that the root exudates of *W. trilobata* help it to spread by suppressing resident pathogens and promoting its invasion.

Keywords: antifungal ability, invasive species, phytopathogenic enzymes, allelochemicals, soil fungi pathogens

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Introduction

Biological invasion is recognized as a major global environmental problem. It can alter ecosystem processes, threaten biodiversity, and cause economic problems [1, 2]. Invasive plants have competitive advantages over neighbouring native species and decrease the biodiversity of the local community [3-5]. One important factor of the highly competitive nature and broad ecological adaptability found in invasive species is allelopathy. Allelochemicals produced by invasive plants can inhibit the growth of competing vegetation by releasing phytotoxic allelochemicals through leaf extracts [6, 7], volatile organic compounds [8], and root exudates [9], thereby providing a competitive advantage for the invader [9]. The novel weapon hypothesis (NWH), which was initially based on the study of diffuse knapweed (*Centaurea diffusa Lam*.) and spotted knapweed (*Centaurea maculosa Lam*.), argued that invaders possess novel secondary metabolites that are not found in native plants and may be toxic to native species [8, 10-12]. There is increasing evidence that allelopathy contributes to the invasiveness of certain plants. For example, (±)-catechin, produced by the invasive *Centaurea maculosa* in the rhizosphere, reduces the growth of native plant species from the invaded range [13, 14]. Some studies have suggested that (±)-catechin might affect bacterial composition and activities in the root [15, 16]. Another allelochemical, o-coumaric acid, has a strong allelopathy effect on the growth of *Arabidopsis thaliana* and other crops [17, 18].

Today, allelopathic effects of invasive species on co-occurring native plants are widely recognized as the major factors responsible for successful invasion in plant communities [19]. However, many previous studies investigated invasive plants' allelopathic potential and impact on native communities [20, 21]. Studies are going on the role soil properties and microbial community play in mediating the impact of invasive species on plants [22, 23]. Various experiments have shown that several invasive flora species may alter soil microbial communities by accumulating or inhibiting pathogens and promoting their further invasion [24-26]. New evidence suggested that invasive plant species may alter the composition and function of soil communities through antimicrobial root exudates [26-29]. The effects of root exudates of non-native plants on soil chemistry, biology, and ecology have gained considerable interest in recent years. Therefore, studying the effects of allelochemicals from invasive plants on soil microorganisms improves our understanding of plant invasion mechanisms. Yet, we have limited knowledge of the relative contribution of direct and indirect allelopathic effects between invasive and other species. Addressing the effects of an alien invasion on soil biota may improve our understanding of the key role soil organisms play in ecological systems, mediating biotic interactions.

Wedelia trilobata (L.) Hitchc. (Asteraceae,), native to tropical America is a representative clonal creeping herb with strong stolons and entomophilous (bisexual) flowers [30, 31]. It was first introduced in Hong Kong and in the 1970s in China [32, 33]. It was also generally used as a ground cover in South China; however, due to its powerful capacity of expansion, *W. trilobata* often overgrows into thick ground cover, crowding out and preventing the regeneration of other plant species [34], resulting in the homogenization of the biota in all ecosystems [35]. Previous studies indicate that *W. trilobata* rapidly spreads through the elongation of clonal ramets and by rooting at stem nodes in contact with soil [36-38]. Aqueous extracts of *W. trilobata* can reduce the germination and growth of local plants [39-41]. Leaf litter of *W. trilobata* inhibit the growth of neighbouring species from enhancing its competitive ability during the early stages of invasion [38]. Additionally, *W. trilobata* leaf extracts promoted the growth of adventitious roots of their own [42]. The volatile oil of *W. trilobata* can also inhibit the mycelial growth of Rhizoctonia solani [43], and its crude extracts also have antimicrobial activities [44]. Component analysis of the extracts of *W. trilobata* has also been conducted in many studies [45, 46]. However, the disease resistance capacity of the invasive plant relative to the indigenous plants is continued.

The disease resistance capacity of *W. trilobata* is relatively higher than the indigenous plants, which increases its resistance to generalist herbivores and pathogens and may give exotic plants an advantage over native species [47, 48]. Our previous field survey showed that almost no disease infection affected *W. trilobata* in 40 randomly sampled plots, which could not be sufficiently explained at present [47]. *W. trilobata* has well-developed roots; in this way, whether and how the root exudates of *W. trilobata* affect soil-borne pathogens through allelopathy has not yet been investigated. Therefore, we hypothesized that the root exudates of *W. trilobata* exhibit a strong allelopathic influence that suppresses soil-borne pathogens. We conducted a series of experiments and set our objectives as (1) to investigate the effect of root exudates of *W. trilobata* on soilborne pathogens, (2) to find out the direct evidence for root exudates of *W. trilobata* as allelochemicals, and (3) to elucidate the antifungal properties of the root exudates. We assessed their effects on the soil-borne pathogen mycelium growth, spore germination, enzymatic (including pectinase, cellulase, and amylase) activities of several phytopathogens, and toxin production.

Materials and Methods

Host Plant and Soil-Borne Phytopathogens

W. trilobata was obtained from Haikou, Hainan, China. Tomato (*L. esculentum* Mill, Qianhong No.1) seeds were obtained from Zhejiang Garden Development Co., Ltd (Hangzhou, China). Rhizoctonia solani (*R. solani*) was purchased from the Agricultural

Culture Collection of China. Fusarium oxysporum f. sp. vasinfectum (F.O. vasinfectum), Sclerotinia sclerotiorum (*S. sclerotiorum*), Phytophthora capsici (*P. capsici*), Fusarium oxysporum f. sp. Melonis (F. oxysporum M), and Fusarium oxysporium f. sp. niveum (F. oxysporium N) were supplied by Zhenjiang Institute of Agricultural Science.

Collection, Purification, and Condensation of Root Exudates

Root exudates were collected using the modified method of Tsanuo et al. [49]. The stem segments of *W. trilobata* were cut into two sections and placed in distilled water until they grew roots. Stem segments with grown roots were transferred into a plastic board [50 cm × 60 cm, perforated with holes (1 cm wide and 5 cm long), each hole is 5 cm apart] and placed on a perspex tray (51 × 61 × 10 cm^3) containing 4 L 0.5 × Hoagland's nutrient solution [50] at pH = 6.0 and 28ºC in a greenhouse. The photoperiod was maintained at a photosynthetically active radiation flux (3870 lux) for 16 h. The stem segments' roots emerged into the nutrient solution from the tray's bottom side. The root exudates and residual media mixture were collected every 5d to 7d.

All collected liquid was instantly filtered through filter paper (quantitative, Wohua Co.). The filtrate was concentrated using a vacuum rotary evaporator at 45ºC. The condensed liquid was mixed with equal amounts of ethyl acetate and allowed to settle for 5 min. The upper organic phase was then separated, and the ethyl acetate extraction process was repeated thrice. The organic phases were combined, dried, weighed, and dissolved with ethyl acetate to prepare stock solutions of root exudates stored at 4ºC [51].

Measurement of Soil-Borne Pathogen Growth under Petri Dish Culture

To assess whether root exudates of *W. trilobata* inhibit the growth of six common soil-borne pathogenic fungi, treatments of root exudates at different concentrations (i.e., 0, 63, and 126 mg/L concentrations) were tested. Each treatment had three replicates. All the chemicals were filter-sterilized using a 0.22 μm Millipore membrane before being added into the steam-sterilized media. As described above, a 5-mm agar plug of each soil-borne pathogen, taken from a 7-day-old potato dextrose agar (PDA) culture, was inoculated at the centre of the plate and incubated at a suitable temperature [52, 53]. The colony area was estimated using Image J software (http://rsbweb.nih.gov/ij/) after the control plaque area of approximately 12 cm^2.

Measurement ofActivities of *R. solani* under Sand Culture

To determine whether *R. solani* inhibits the growth of *W. trilobata*, stems of *W. trilobata* without roots were planted in sterile sand (121ºC, 120 min), and then 50 g of the sand was placed in a 50 ml centrifuge tube. Two 5-mm agar plugs of *R. solani* were taken from a 7-day-old PDA culture were introduced into each tube. For no-pathogen treatments, each tube received two same-sized pathogen-free agar plugs. Each tube received 10 ml 0.5 × sterilized Hoagland's nutrient solution. Each treatment had nine replicates, randomly placed in an illuminated greenhouse (3870 lux, photoperiod was 16 h) at 28ºC, grown for 12 d [54, 55], and the relative increase in the amount of fresh weight was measured. The rate of the relative increase in fresh weight was calculated as follows;

$$Rate\ of\ relative\ increase\ (\%) = \left(\frac{fresh\ weight}{background\ value} - 1\right) \times 100 \quad (1)$$

To determine whether cultured *W. trilobata* significantly inhibited *R. solani*, we placed 400 g of the sterilized sand culture in 40 pots (7 cm wide × 9 cm deep). Stems of *W. trilobata* were planted into twenty pots (treatment), and the remaining pots were left without planting *W. trilobata* stems (CK). All pots were randomly placed in an illuminated greenhouse (3870 lux, photoperiod was 16 h) at 28ºC. Next, 20 ml 0.5 × sterilized Hoagland nutrient solution was poured once a week. After five months, individuals of *W. trilobata* were removed, and soil-borne pathogen RS was inoculated in five treatment pots (grown *W. trilobata*) and five CK pots (without grown *W. trilobata*). For no-pathogen treatments, each pot received the PDA without *R. solani* with a size similar to that for those with the pathogen. Twenty tomato seeds were surface-sterilized with H_2O_2 (5%, v: v) before being planted into each pot. After the tomatoes were sown, 0.5 × sterilized Hoagland's nutrient solution was added to maintain the normal growth of the seedlings. The number of germinated seeds was recorded after one month. The survival rate was calculated as follows;

$$Survival\ rate\ (\%) = \frac{number\ of\ living\ seedlings}{number\ of\ sown\ seeds} \times 100 \quad (2)$$

Two treatments of 0 and 200 mg/L of root exudates were tested to determine the impact of root exudates of *W. trilobata* on *R. solani*, and its growth, The control (0 mg/L) was obtained by adding an equivalent volume of DMSO, a latent solvent, and 0.5 × sterilized Hoagland's nutrient solution. First, 50 g of sterilized sand culture was placed in a 50 ml centrifuge tube. Then, two agar plugs (0.5 cm wide) of RS taken from a 7-day-old PDA culture were introduced into each tube. Stems of *W. trilobata* without roots were planted in the sterile sand culture. Each tube received 10 ml of the corresponding concentration of root exudates. Each treatment had nine replicates, randomly placed in an illuminated greenhouse (3870 lux, photoperiod was 16 h) at 28ºC, grown for 12 d, and the relative increase in the amount of fresh weight and root length was measured.

Inhibition Mechanism of Root Exudates on Soil-Borne Pathogens

Three common soil-borne pathogens *R. solani*, *S. sclerotiorum*, and F. oxysporum were used in the following experiments to test the effects of *W. trilobata* root exudates on the *R. solani* and *S. sclerotiorum* mycelial dry weight and enzymatic activities of pectinase, cellulase, and amylase. The effects of root exudates on the mycotoxin of oxalic acid produced by *S. sclerotiorum* and the conidia germination ratio of F. oxysporum were also determined.

Inhibition Effect of Root Exudates on Fungal Biomass

Five treatments (0, 25, 100, 200, and 300 mg/L) of root exudates were used to check the Inhibition effect of root exudates on fungal biomass. The control (0 mg/L) was obtained by adding an equivalent volume of ethyl acetate. Each treatment had five replicates. All the chemicals were filter-sterilized using a 0.22 μm Millipore membrane before being added into the steam-sterilized media. The medium for the biomass production assay and the pathogen's enzymatic activity was based on Marcus's culture medium [56] with minor modifications. It consisted of 1.0 g K_2HPO_4, 2.0 g KNO_3, 0.5 g KCl, 0.01 g $FeSO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g L-asparagine, 0.2 mg vitamin B_1, 10.0 g carboxymethylcellulase (CMC), and 1000 ml distilled water at pH = 5.0. The *R. solani* and *S. sclerotiorum* were grown in 50 ml conical flasks with 10 ml culture medium inoculated with a same-sized agar plug taken from a 7-day-old PDA culture of *R. solani* and *S. sclerotiorum*. The *R. solani* cultures were incubated and shaken (180 rpm) at 28ºC for 7 d. The *S. sclerotiorum* cultures were incubated at 25ºC. Fungal biomass (dry weight) was determined after filtration with quantitative filter paper and drying at 80ºC for 12 h until a constant weight was achieved. The culture filtrate was used for the enzymatic and mycotoxin assays [51].

Effect of Root Exudates on Enzymatic Activities

Cellulase activity was assayed using the 3,5-dinitrosalicylic acid (DNS) method [57, 58] with little modification. The reaction system, containing 0.25 ml of the above enzyme extract and 0.75 ml 0.5% (w/v) CMC solution, was incubated at 50ºC for 30 min. Then, 1.5 ml DNS was added into the system and thoroughly mixed. The mixture was heated in the water bath at 100ºC for 10 min and then cooled. Next, 10 ml of Wahaha purified water was added. The mixture was colorimetrically determined (A540) using a u.v. spectrophotometer (UV-1200 spectrophotometer Mapada). One unit of enzymatic activity was defined as the amount of reduced sugar from CMC per hour per 1 ml crude enzyme under the above assay conditions. Glucose (Aladdin Chemistry Co. Ltd) was used to prepare the standard curve under similar conditions. Pectinase activity (mainly polygalacturonase) was assayed using the DNS method [56, 58] with some modifications. The reaction mixture comprised 0.25 ml of the above enzyme extracts and 0.75 ml 0.5% (w/v) pectin (poly-D-galacturonic acid ethyl ester, Aladdin Chemistry Co. Ltd) solution. The mixture was reacted at 50ºC in a water bath for 30 min. Next, 1.0 ml DNS was added to the mixture, which was boiled for 10 min, and 8 ml Wahaha purified water was added after it was cooled. The reduced sugar content of the reaction mixture was colorimetrically determined (A540). Next, a 1% (w/v) b-galacturonic acid standard sample (Aladdin Chemistry Co. Ltd) was used to prepare a standard curve. One unit of enzymatic activity was defined as the amount of b-galacturonic acid hydrolyzed from pectin per minute per millilitre under assay conditions.

Total amylase activity was assayed using the DNS method [59] with some modifications. The mixture comprised 0.25 ml of the above enzyme extract and 0.75 ml of 0.5% (w/v) soluble starch. The reaction mixture was incubated at 37ºC for 30 min. The enzymatic reaction was terminated by adding 1.5 ml DNS and boiling for 10 min. Finally, 10 ml of Wahaha purified water was added after it was cooled, and the released reduced sugar was colorimetrically determined (A540). One unit of enzymatic activity was defined as the amount of reduced sugar from soluble starch per hour per millilitre of the crude enzyme under the above assay conditions. Amylomaltose (Aladdin Chemistry Co. Ltd) was used to prepare a standard curve.

Inhibitory Effects of Root Exudates on Conidia Germination

Five treatments (0, 25, 100, 200, and 300 mg/L) of root exudates were used. A F. oxysporum spore suspension (8.95×10^5 spores/ml) was incubated with germination solution (40% PDA without agar, different concentrations of root exudates) in a total volume of 200 μl in a 96-well plate in the dark at 28ºC. After 8 h, the number of spore germination was counted under the microscope.

Inhibitory Effect of Root Exudates on Mycotoxin Production

Mycotoxin of oxalic acid was assayed using the spectrophotometric coloration method [60]. The mixture comprised 0.5 ml 0.5 mg/ml $FeCl_3$, 5 ml KCl buffer solution (pH = 2.0), 0.3 ml 0.5% (w/v) salicylsulfonic acid, 1 ml oxalic acid extract, and 3.2 ml Wahaha purified water. The reaction mixture was incubated at room temperature for 30 min, and the oxalic acid was colorimetrically determined (A510).

Statistical Analysis

All statistical analyses were performed with SAS software (version 9.1) (SAS Institute Inc. 2004). Duncan's multiple range test was applied after one-way ANOVA revealed significant differences ($P \leq 0.05$). The values were represented as means of three or five replicates (mean±SD) for each treatment, and SD means standard deviation. Interventional studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

Results and Discussion

Measurement of Soil-Borne Pathogen Growth under Petri Dish Culture

Root exudates of *W. trilobata* significantly inhibited the growth of six pathogenic fungi to different extents. The six pathogenic fungi growths were significantly suppressed in solid culture ($P<0.05$, Table 1). The growth of *R. solani* was 26.15% at 126 mg/L root exudate concentration compared to the control. Similarly, the growth of F.O. vasinfectum, *S. sclerotiorum*, *P. capsici*, F. oxysporum M, and F. oxysporium N was decreased to 29.45%, 36.34%, 13.05%, 30.81% and 14.31%, respectively (Table 1). The Novel Weapons hypothesis suggests that invasive plants can secrete secondary metabolites into the soil subsystem from their roots as exudates and may alter the composition, as well as function, of the soil community [61, 62]. These allelochemicals are also implied in defence against predators and plant pathogens [63, 64]. Results from our experiments (Petri dishes culture) showed that W. trilobata root exudates inhibited the colony growth of six common soil-borne fungal pathogens, implying that W. trilobata could affect soil-borne fungal pathogens through allelopathy. However, this study has not investigated the chemical structure of these allelochemicals. Our further experiment in sand culture demonstrated that *W. trilobata* root exudates inhibited the activity of *R. solani* (using the survival rate of tomato seedlings as an indicator) through allelopathy and facilitated its growth. These findings are consistent with those of studies on the allelopathic effects of secondary metabolite in other plant species. For example, Kato et al. [65] found that *S. canadensis* had allelopathy on population growth and pathogenic activity of soilborne pathogens. Additionally, it was noted in many studies that the accumulation of secondary metabolites in soil contributes to the allelopathic effects of *S. canadensis* suppressing soil-borne pathogens [10, 66-68].

Table 1. Effect of *W. trilobata* root exudates on the colony growth of *R. solani*, *S. sclerotiorum*, *P. capsici*, F.O. vasinfectum, F. oxysporum M, and F. oxysporium N in a solid culture medium. Error bars represent the standard deviation of three replicates.

Fungus	Concentration of exudate (mg/L)	Area of colony		Area change (%)
		Avg. cm^2	SD	
R. solani	0	9.98a	0.40	
	63	7.82b	0.46	-21.60
	126	7.37b	0.32	-26.20
S. sclerotiorum	0	13.15a	1.22	
	63	9.70b	1.76	-26.24
	126	8.37b	0.78	-36.32
P. capsici	0	12.10a	0.53	
	63	11.82a	0.45	-2.30
	126	10.52b	0.12	-13.02
F.O. vasinfectum	0	12.02a	0.43	
	63	9.09b	0.32	-24.39
	126	8.48b	0.40	-29.42
F. oxysporum M	0	10.84a	0.18	
	63	9.89b	0.78	-8.77
	126	7.50c	0.19	-30.79
F. oxysporium N	0	11.32a	0.91	
	63	10.37ab	0.62	-8.42
	126	9.68b	0.20	-14.53

Measurement of Activities of *R. solani* under Sand Culture

Under pathogen treatment, the growth of *W. trilobata* stems was significantly affected by *R. solani* under sand culture ($P<0.05$). The rate of increase in the fresh weight of the stems of *W. trilobata* under the no-pathogen treatment was significantly higher than that under the *R. solani* treatment ($P<0.05$, Fig. 1a). Both pathogen and plant significantly affected the survival rate of the tomato ($P<0.05$, Fig. 1b). Under no-pathogen treatments, the survival rates of the tomato were significantly lower in treatments with *W. trilobata* than that in the treatment in which no *W. trilobata* was planted (control) ($P<0.05$, Fig. 1b). This result suggests that *W. trilobata* had allelopathic effects on tomato seedlings. However, the treatments of *W. trilobata* + *R. solani* significantly increased the survival rates of the tomato compared with the treatments with pathogens only (no plants grown before), suggesting that *W. trilobata* inhibited *R. solani* via secondary metabolites. Root exudates significantly affected the *W. trilobata* fresh weight increase and root length ($P<0.05$).

Soil-borne pathogen *R. solani* is commonly found in agricultural soils in southern China [69, 70]. Thus, we used *R. solani* in our experiments. While, the experiment in sand culture demonstrated that *R. solani* inhibited the growth of *W. trilobata* (Fig. 1a). To determine the separate effects of allelopathic from other soil factors by growing *W. trilobata* under sterilized sand with inoculated *R. solani*, we designed an experiment. The results demonstrated that the pathogenic activities of *R. solani* were inhibited under *W. trilobata* (indicating by the survival rate of tomato) (Fig. 1b). *W. trilobata* can secrete allelochemicals into the rhizosphere and affect the activities of pathogens. The activity of microorganisms in the rhizosphere

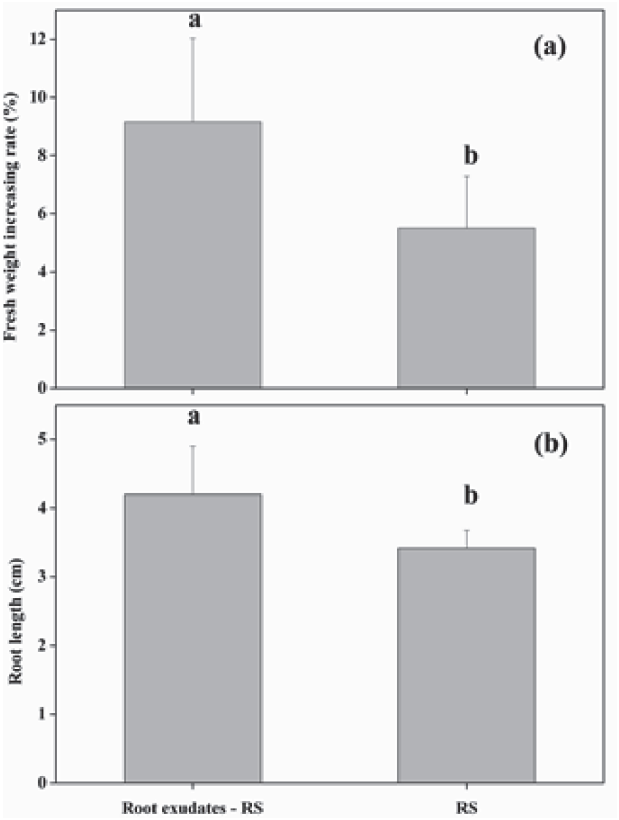

Fig. 2. Effects of root extracts of *W. trilobata* on *R. solani* determined by detecting the pathogenic activity through the increasing rate of *W. trilobata* fresh weight and root length under sand culture. Error bars represent standard deviation of nine replicates, data with different superscript letters indicate a significant difference ($P<0.05$).

enhances by the root exudates of invasive species, which give positive feedback to it and support its invasion [71]. *W. trilobata* has also been found to have potential

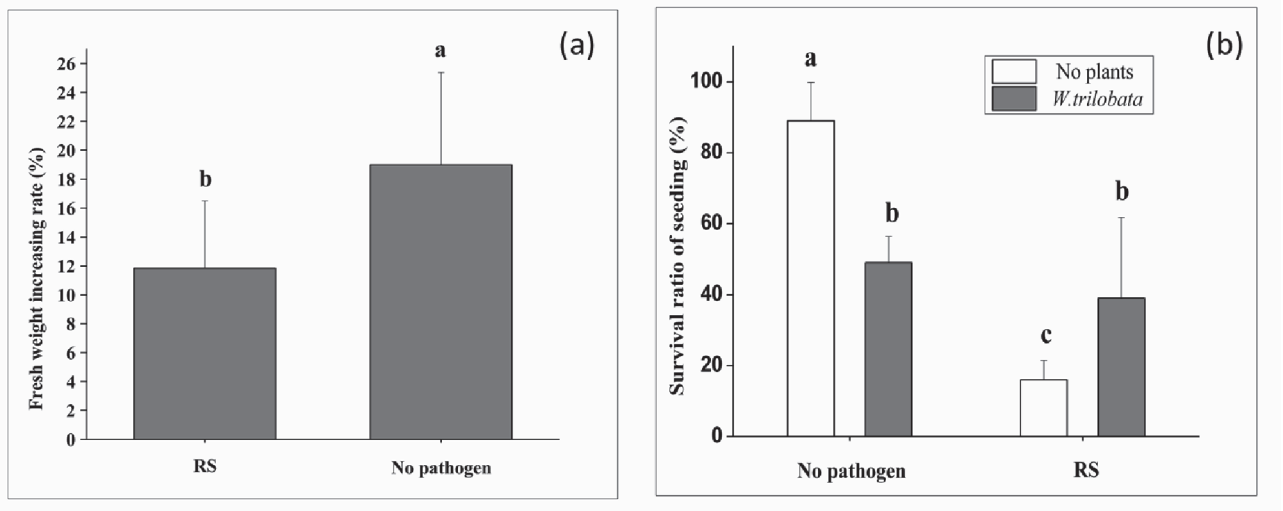

Fig. 1. Influence of *R. solani* on the growth of *W. trilobata* under sand culture a), and effects of root extracts of *W. trilobata* on *R. solani* by detecting the pathogenic activity on the survival rate (%) of tomato seedlings under sand culture b). Error bars represent standard deviation, data with different superscript letters indicate a significant difference ($P<0.05$). In the figure RS represents *R. solani* .

allelopathic effects on rice, tomato, turnip, radish, onion, cabbage, chickpea, cowpea, and green gram [41, 72, 73]. In this study, we also found that *W. trilobata* had allelopathic effects on tomatoes (Fig. 1b). Thus, the results support our hypothesis that *W. trilobata* might affect both on local plants and soil-borne pathogens by secreting allelochemicals. We designed another experiment to prove that root exudates of *W. trilobata* collected with the water culture method exhibit the same effect. The results provided indirect evidence that *W. trilobata* root exudates inhibit *R. solani* (use fresh weight and root length) (Fig. 2). However, the treatments of root exudates + *R. solani* significantly increased the increasing rates of *W. trilobata*'s fresh weight (P<0.05, Fig. 2a) and root length (P<0.05, Fig. 2b), suggesting that the root exudates of *W. trilobata* suppressed *R. solani* and facilitated its growth.

Inhibition Mechanism of Root Exudates on Soil-Borne Pathogens

Inhibition of *R. solani* and *S. sclerotiorum* growth by root exudates was evident even at the 300 mg/L root exudate concentration. *R. solani* and *S. sclerotiorum* growth were strikingly suppressed at 7 d incubation in the liquid culture. The dried weights of *R. solani* and *S. sclerotiorum* mycelia in the 300 mg/L treatment were lower than that of the control (Fig. 3a). The root exudates of *W. trilobata* were found to suppress soil-borne fungal pathogens. Root exudates have two main effects on soil-borne fungal pathogens. One effect includes the inhibition of hyphal growth and conidial germination. In contrast, the other involves inhibiting mycotoxin production and enzymes related to pathogenesis [74], representing the suppression mechanism of *W. trilobata* root exudates on the activities of soil-borne pathogens.

Root exudates significantly affected cellulase activities in liquid culture (P<0.05). The cellulase activities of *R. solani* and *S. sclerotiorum* from the treatment without root exudates to the condition under 300 mg/L root exudate concentration decreased from 1.70±0.11 units/ml to 0.03±0.01 units/ml and 1.26±0.09 units/ml to 0.008±0.014 units/ml (Fig. 3b), respectively. Root exudates significantly decreased pectinase activities in the liquid culture (P<0.05). The activities of *R. solani* and *S. sclerotiorum* at concentrations of 0 mg/L to 300 mg/L decreased from 10.81±0.94 units/ml to 3.06±0.35 units/ml

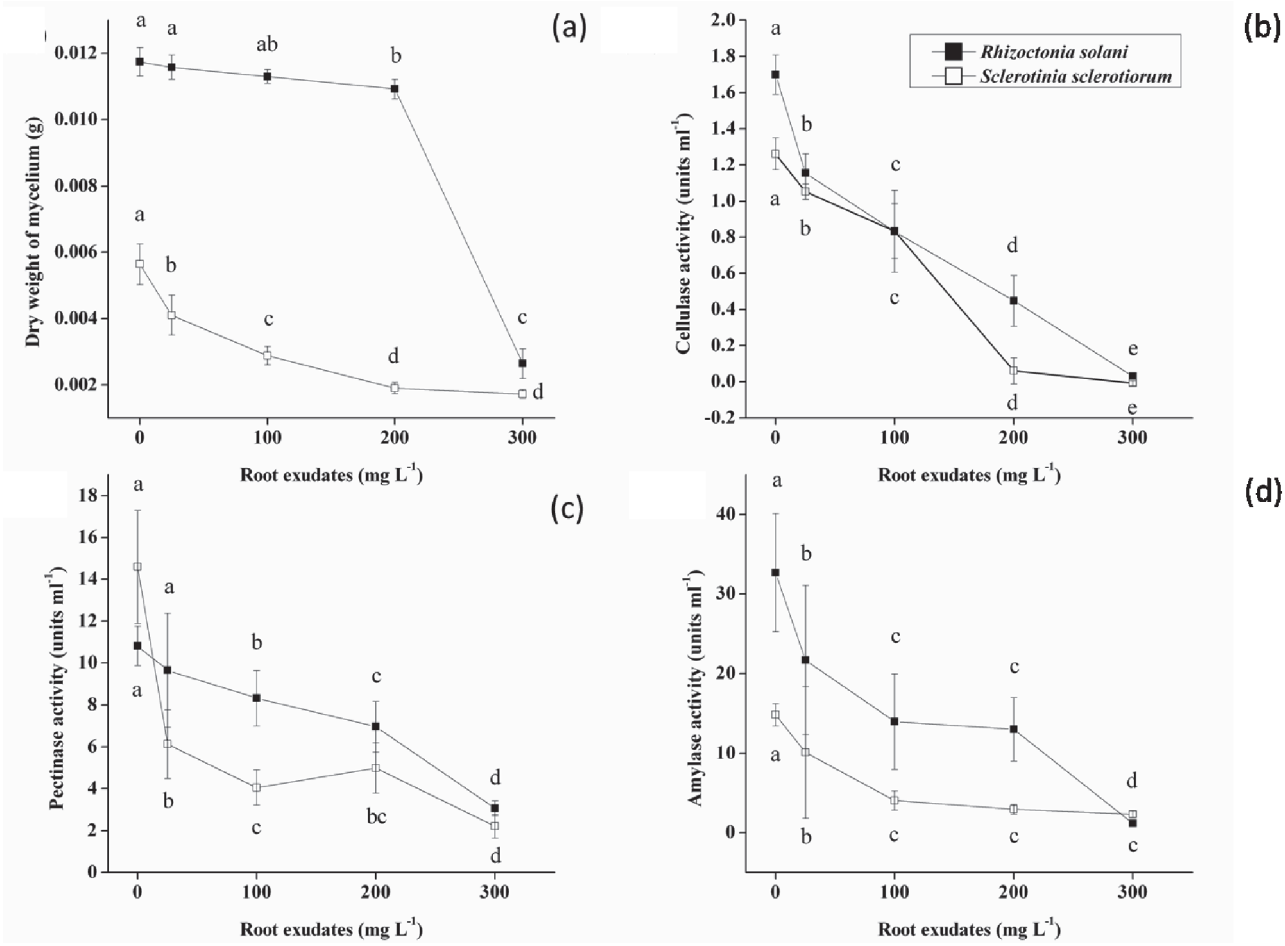

Fig. 3. Influence of root exudates on the enzymatic activities and biomass productions of *R. solani* and *S. sclerotiorum* in a liquid culture after 7 d incubation. Error bars represent standard deviation of five replicates, data with different superscript letters indicate a significant difference (P<0.05). In the figure RS represents *R. solani* and SS represents *S. sclerotiorum*.

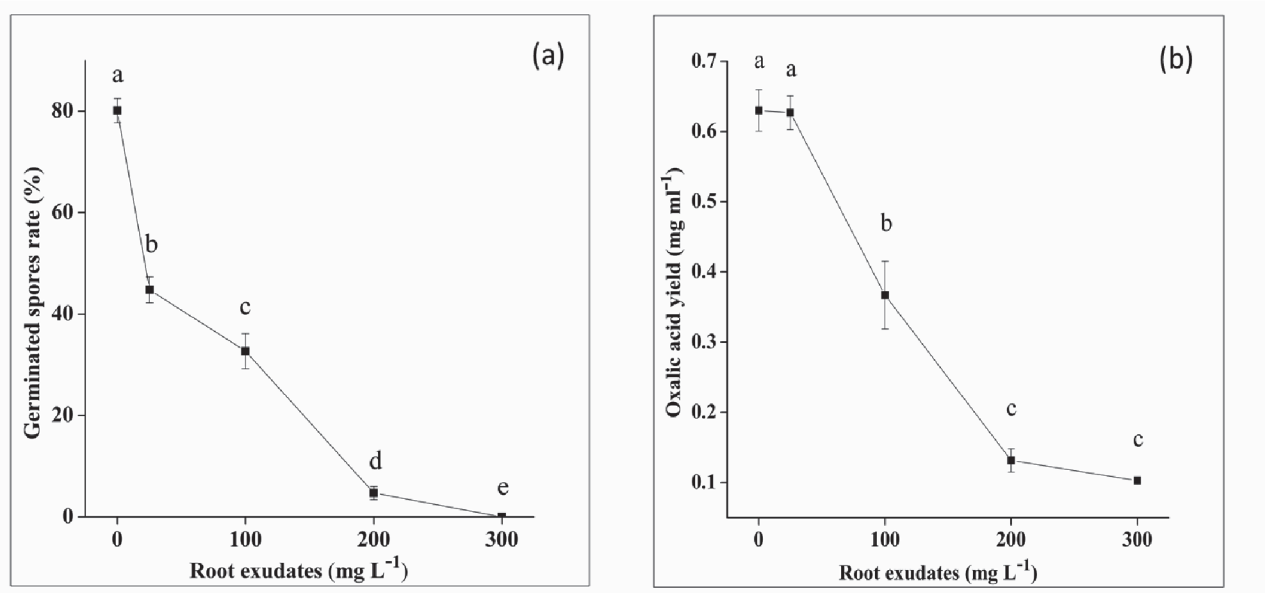

Fig. 4. Influence of root exudates on the spore germination of F. oxysporum M in a liquid culture after 8 h a), and Influence of root exudates on mycotoxin (oxalic acid) production of *S. sclerotiorum* in a liquid culture b). Error bars represent standard deviation, data with different superscript letters indicate a significant difference (P<0.05).

and 14.60±2.70 units/ml to 2.21 to 0.56 units/ml, respectively (Fig. 3c). Root exudates significantly affected amylase activities in liquid culture (P<0.05). The amylase activities of *R. solani* from the treatment without root exudates to the condition with 300 mg/L root exudates decreased from 32.66±7.44 units/ml to 1.17±0.20 units/ml (Fig. 3d). The amylase activity of *S. sclerotiorum* decreased from 14.80±1.39 units/ml to 2.31±0.38 units/ml (Fig. 3d). Our results demonstrated that root exudates strongly inhibited *R. solani* and *S. sclerotiorum* hyphal growth and biomass. The lowest dry weight of mycelia was obtained in the liquid culture with the highest concentration of root exudates (300 mg/L) (Fig. 3). Hence, the root exudates enhanced plant resistance to the pathogen and directly inhibited the growth of the pathogen. The root exudates were unfavourable for *R. solani* and *S. sclerotiorum* because they suffered multiple suppressions, including hyphal growth, biomass, and colony growth.

All hyphal growth and conidial germination are important preconditions for the survival and attack of pathogenic microorganisms on their hosts [75]. The spores of F. oxysporum were easy to obtain and used to assess the effects of root exudates on conidial germination [76]. In the present study, the conidial germination of F. oxysporum in liquid culture was markedly suppressed by root exudates even at the lowest concentration of 25 mg/L. Conidial germination was inhibited in the liquid culture with the highest concentration of root exudate (Fig. 4a). Severe repression of conidia germination by F. oxysporum was obtained in a concentration-dependent manner. Dramatic inhibitions of conidia germination were found at all concentrations (25 to 300 mg/L), especially at 200 and 300 mg/L, in which germination was inhibited completely (Fig. 4a).

The mycotoxin yield of *S. sclerotiorum* in liquid culture was decreased by root exudates and further decreased with increasing concentrations. Mycotoxin production decreased remarkably at high concentrations (200 and 300 mg/L). The yields at different treatments (0 to 300 mg/L) decreased from 0.6±0.048 mg/ml to 0.10±0.003 mg/ml (Fig. 4b). The fatal pathogenic factor of mycotoxin causes plants to wilt. Oxalic acid is likewise known to play a key role in the pathogenesis produced by *S. sclerotiorum* [77]. In this study, root exudates inhibited mycotoxin production by *S. sclerotiorum*. At the highest concentration of root exudates, mycotoxin yield decreased by 83.2% (Fig. 4b). Hydrolytic enzymes are other important phytopathogenic factors for some soil-borne pathogens. In many plant diseases, pectinases and cellulases of phytopathogenic fungi can promote the infection process and promote the penetration of a fungus into the plant through hydrolytic cleavage of polymers (such as pectic substances and cellulase), which constitute important constituents of the plant cell walls [78, 79]. In the present study, root exudates significantly inhibited the enzymatic activities of *R. solani* and *S. sclerotiorum* in the liquid cultures and at the highest concentration (300 mg/L), cellulase activities declined by 98.1% and 100% (Fig. 3) compared with the control. Pectinase activities produced by *R. solani* and *S. sclerotiorum* at the highest concentration of root exudates decreased by 71.7% and 84.9% (Fig. 3), respectively. Amylase activities also decreased following treatment with root exudates (Fig. 3). Therefore, our results suggested that root exudates strongly inhibited soil-borne pathogens' growth, conidial germination, mycotoxin production, and amylase activities.

Conclusions

We noted that these root exudates are one of the plants' main mechanisms in inhibiting soil-borne pathogens through root exudates. The results showed that the root exudates of *W. trilobata* significantly inhibited the growth and activities of soil-borne pathogens under sterile petri dish and sand cultures. Root exudates significantly inhibited the enzymatic activities of *R. solani* and *S. sclerotiorum* in the liquid cultures, and at the highest concentration (300 mg/L), cellulase activities declined by 98.1% and 100%. However, in this study, we have only investigated root exudates extracted by ethyl acetate. The role of root exudates in the aqueous phase was not determined. The chemical structure of root exudates of *W. trilobata* was likewise not investigated in this study. Therefore, further study on the molecular mechanism of growth inhibition by allelochemicals is the prospect, and it is needed to determine the major impact of allelochemicals released by invasive species and their interactions with the soil environment.

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Conflict of Interest

The authors declare no conflict of interest.

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