

## Fungicidal effects of wheat root exudates on *Fusarium oxysporum* f. sp. *niveum*

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### ABSTRACT

Root exudates from 9-wheat varieties were assayed for their fungicidal effects on growth of *Fusarium oxysporum* fs.p. *niveum*. The exudates of all 9-varieties inhibited the mycelial growth to varying degrees, however, the root exudate of variety L-16 was most inhibitory. The root exudate of variety L-03K756 was most inhibitory to spore germination of fungus. The chemical analysis of root exudates of 9-wheat varieties with GC-MS showed that the composition of root exudates, varied with the variety and contained 30 different organic compounds, some of which are known allelochemicals. The hexadecanoic acid in wheat root exudates inhibited the *Fusarium oxysporum* fs.p. *niveum* especially at 1mmol·L<sup>-1</sup>.

**Key words:** Allelochemicals, *Fusarium oxysporum* f. sp. *niveum*, GC-MS, hexadecanoic acid, root exudates, wheat

### INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *niveum* is often observed in watermelon under monocropping and results in yield losses (33). Once in the watermelon soil, this pathogen is very difficult to eradicate (16) and infects the host plants in all growing stages (29). To control and prevent its spread, many countries including China, have recognized the need to develop eco-friendly bio pesticides and related technologies including the use of allelopathic crop plants (11).

It is known that in mixed cropping or intercropping, the root exudates exert their allelopathic effects, which initiate and manipulate the biological and physical interactions between the roots and soil organisms, decreases the crop disease incidence and increase the crop yields (9,12,20). The intercropping of wheat and cucumber improves the soil micro flora and reduces the number of soil-borne pathogens, especially *Fusarium oxysporum* in cucumber (15,22). Recent studies with wheat suggest that wheat root exudates also inhibits the growth of *Fusarium* (10). However, the reasons for these effects are not clear. This study aimed to explore the effects of 9-wheat varieties root exudates on the growth of *F. oxysporum*. The root exudates inhibited the growth of *F. oxysporum*, hence, were analysed by GC-MS to determine the allelochemicals.

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## MATERIALS AND METHODS

### Plant material and fungal strain

*Fusarium oxysporum* f.sp. *niveum* physiological race No. 1 was isolated from diseased watermelon plants in Heilongjiang Province, and stored at 4°C in refrigerator on Potato Dextrose agar (Potato 200g, glucose 20g, water 950mL, agar 20g) slants by regular transfer. Nine wheat varieties (L-18, L-17, L-16, L-7, L-03K756, D-05-4288, D-125, D-08-8012 and D-D) were provided by Laboratory of Horticultural and Vegetable Physiology and Ecology in our University. Hexadecanoic acid (AR) was bought from Better Chemicals, Shanghai.

### Collection and processing of root exudates

Healthy and uniform sized wheat grains were carefully selected. Soaked, primed and then sown in seedling trays (90cm× 60cm × 12cm) containing the substrate (peat: vermiculite = 1:1). Fifteen days after emergence, the seedlings were taken out of the nursery substrate avoiding root damage. The seedlings were washed several times with tap water, 4 times with distilled water and finally twice rinsed with deionized water. One hundred and twenty seedlings were placed in 100 ml-flasks containing 40 ml distilled water. The flasks were placed in normal dark and light conditions. After 2 days, the water in the flasks containing the root exudates was filtrated through 3 layers of filter paper in a Buchner funnel, and then through a 0.45 µm membrane. After filtration, the root exudates were stored in refrigerator at 4 °C. The root exudates were lyophilized for 16 h to get the dry crude extract before the test. Then, the dry residue of each root exudates was dissolved in water to prepare a concentration of 0.1 g·ml<sup>-1</sup> (7,10).

### Effects of root exudate of wheat var.L-03K756 on *F. oxysporum* growth

The root exudates of wheat variety L-03K756 was assayed in 90 mm dia Petri dishes at 0, 0.025, 0.05, 0.1 g·ml<sup>-1</sup> concentrations. In each Petri dish, 19 ml of autoclaved PDA medium (at 50 °C was mixed with 1 ml of root exudate (0.1g·ml<sup>-1</sup>) of wheat variety L-03k756. After cooling, each Petri dish was centrally inoculated with 5-mm agar plug obtained from a 7-day-old colony of *F. oxysporum* also grown in PDA (10).

Controls contained 1 ml of sterile distilled water added to the PDA medium instead of root exudate. Each treatment was replicated thrice. The Petri dishes were placed in an incubator at 28 °C for 5 days and thereafter the colony diameter was measured in two directions at right angles (7,10).

### Effects of root exudate from wheat var L-03K756 on spore germination

To determine the effect of wheat root exudate on conidial germination, 1 ml root exudate of wheat var. L-03K756, was mixed with 19 ml of 2% water agar at 55 °C and poured into a 90 mm Petri plate. A 5-mm agar plug taken from a 7-day-old PDA culture was inoculated into 150 ml conical flask containing 50 ml-liquid Potatoe dextrose medium and incubated at 28 °C for 7d. The liquid culture was filtered to collect the conidia. Conidial suspension was diluted to 1000 conidia per ml with sterile distilled water. The diluted suspension (0.1 ml) was spread on 2% water agar containing root exudate and incubated at 28 °C for 3d and the number of colonies determined (23).

### Effects of hexadecanoic acid on *F. oxysporum* growth

Hexadecanoic acid at 0, 0.001, 0.01, 0.05, 0.1 mmol was dissolved in 1.5 ml acetone, the solution was filter-sterilized and then added at 100 ml to autoclaved PDA medium. Hexadecanoic acid at 0, 0.01, 0.5, 1 mmol·L<sup>-1</sup> was used to determine the colony diameter of *F. oxysporum*, 0 being control. The PDA medium was centrally inoculated with 5-mm agar plug obtained from a 7-day-old colony of *F. oxysporum* also grown in PDA (10). Each treatment was in triplicate. The Petri dishes were placed in an incubator at 28 °C for 5 days and thereafter, the colony diameter was measured in two directions at right angles after 2, 3, 4 and 5 d (7,10).

### Effects of root exudates from 9-wheat varieties on *F. oxysporum* mycelial growth rate

The root exudates from nine wheat varieties were assayed in 90 mm-diameter Petri dishes at a concentration of 0.1 g·ml<sup>-1</sup>. The plates were incubated at 28 °C for 7 days. The colony diameter was measured daily in two directions at right angles to each other.

Growth rate %, expressed in colony diameters, was calculated as under:

$$W \% = (dn-d1)/d1 \times 100.$$

Where, W: Rate of growth, dn: Colony diameter at n days t and d1: Colony diameter on first day of assay.

### GC-MS analysis of wheat root exudate

Chemical composition of wheat root exudates of the 9-wheat varieties was determined by GC-MS at Institute of Technology with method of yang *et al* (20) using a capillary column DB-5 (5% diphenyl and 95% diphenylsiloxane, 60 m x 0.25 mm, 0.25 µm film). Aqueous root exudate samples (10 ml, at a concentration of 0.1g·ml<sup>-1</sup>) were partitioned with n-butanol: water system, in a ratio of 2:1 (v/v, water: n-butanol). The n-butanol phase was recovered and treated with anhydrous sodium sulphate (5 g) to eliminate the remaining water. The organic phase was filtrated through a 0.22 µm membrane and subjected to GC-MS (GC 6890, MS 5973) analysis. Helium was used as carrier gas at a flow rate of 1 ml/min, and the temperature conditions were as follows: 100 °C for 5 min, from 100 to 140 °C (10 min), 140 °C (5 min), from 140 to 230 °C (15 min), 230 °C (5 min). Inlet : 250 °C; ion source: 230 °C; quadrupole: 150 °C transmission line: 270 °C; ionization mode: EI, electron energy 70 eV; mass scan range: 40 to 500 amu; solvent delay time 6.5 min (25,32). The various components were identified from the retention times using the reference material from Mass spectral Library.

### Statistical Analysis

Data were subjected to ANOVA using SAS 9.13 (SAS, Inc., Cary, USA). The significant differences among treatments were determined by Turkey's test significant difference test at P < 0.05.

## RESULTS AND DISCUSSION

### Effects of wheat var.L-03k756 root exudate on fungal growth

The fungicidal effect of root exudates of wheat var. L-03K756 varied with concentrations on growth of *F. oxysprum* fungus (Fig 1, at 5 d). The growth of fungus was significantly inhibited by the root exudate and the extent of inhibition increased with concentration of root exudate. The higher the root exudate concentration, the smaller was the colony diameter. Significant inhibition was noticed with 1ml root exudates ( $0.1 \text{ g}\cdot\text{ml}^{-1}$ ) and this concentration was used in testing the effects of root exudates of other varieties on fungal growth.

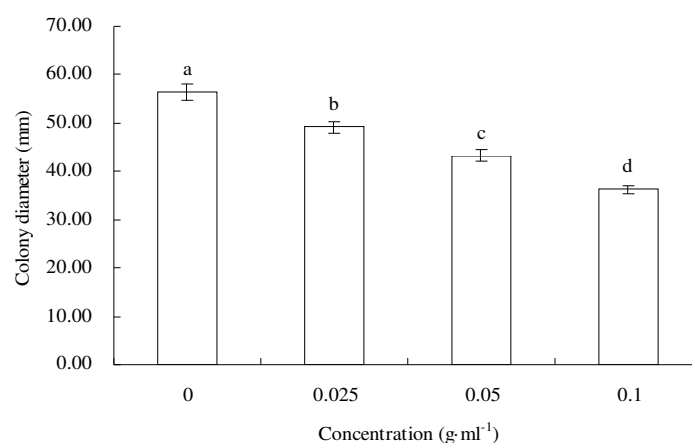


Figure 1. Effects of wheat variety L-03K756 root exudates concentrations [0 (Control), 0.025, 0.05,  $0.1 \text{ g}\cdot\text{ml}^{-1}$ ] on colony diameter of *F. oxysporum* after 5 days. Bars indicate Standard Error. Different letters in the same chart mean significant differences ( $P < 0.05$ , Turkey's test).

### Effects of root exudates on Spore germination

The effects of root exudates of wheat var. L-03k756 on germination of fungal spores and colony formation was determined after 3 days (Fig. 2.) The root exudate significantly inhibited the spore germination. The extent of inhibition increased with the concentration of root exudate and maximum inhibition occurred at  $0.1 \text{ g}\cdot\text{ml}^{-1}$  concentration. Only about 50% of spores germinated at  $0.1 \text{ g}\cdot\text{ml}^{-1}$  concentration.

### Effects of hexadecanoic acid on *F. oxysporum* growth

Effects of different concentrations of hexadecanoic acid were studied on colony diameter of *F. oxysporum* at 2, 3, 4 and 5 d (Fig. 3). At 2d, the effects of hexadecanoic acid treatments were measured on the colony diameter of *F. oxysporum* and were similar to control. However at 3, 4 and 5 d, the colony diameter with  $1 \text{ mmol}\cdot\text{L}^{-1}$  of hexadecanoic acid were significantly lower than control. The other treatments had no significant effects on *F. oxysporum* growth.

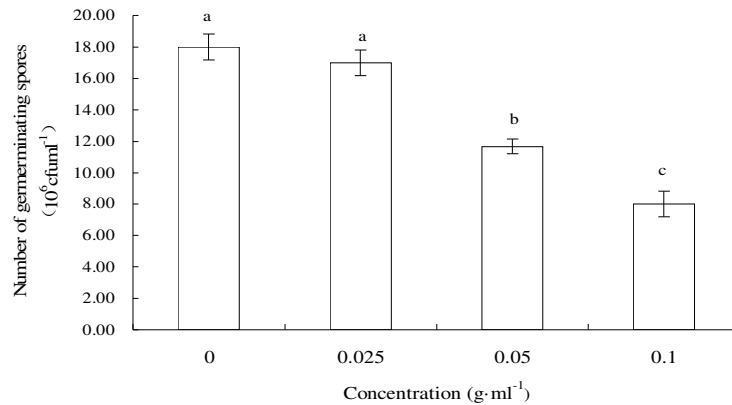


Figure 2. Effects of wheat variety L-03K756 root exudates concentrations [0 (Control), 0.025, 0.05, 0.1g·ml<sup>-1</sup>] on spore germination of *F.oxysprum* after 3days. Bars indicate Standard Error. Different letters in the same chart mean significant differences (P <0.05, Turkey's test).

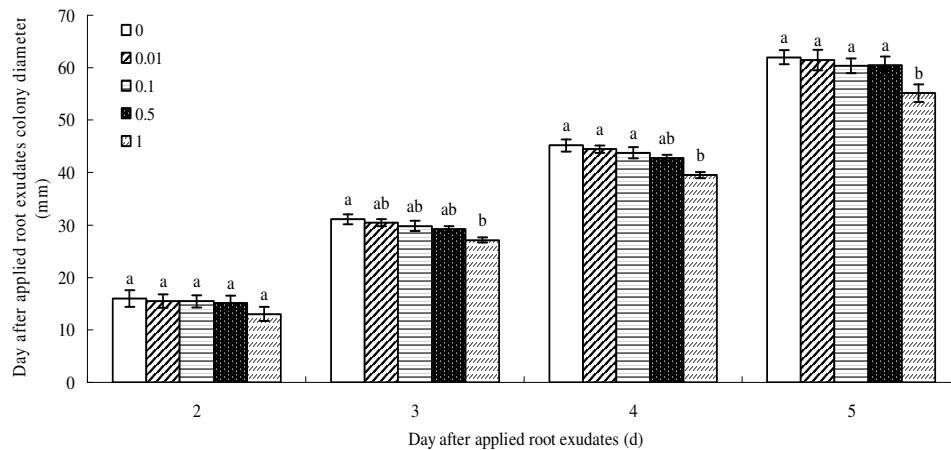


Figure 3. Effects of hexadecanoic acid concentrations [0 (Control), 0.01, 0.1, 0.5, 1mmol·L<sup>-1</sup>] on colony diameter of *F. oxysporum* after 2, 3, 4 and 5 d. Bars indicate Standard Error. Different letters in the same chart mean significant differences (P<0.05, Turkey's test).

#### Effects of 9-wheat varieties root exudates on *F. oxysporum* growth

Root exudates of 9-wheat varieties were tested at 0.1 g·ml<sup>-1</sup> concentration for their effects on growth of *F.oxysporum*.

The mycelial growth was affected by the root exudates to varying degrees (Table 1). The root exudate of variety L-16 exerted the strongest inhibitory effects on growth and the degree of inhibition was 50% of control. The least inhibition was caused by the root exudate of variety D-D.

Table 1. Effects of root exudates from 9-wheat varieties on growth rate of *F. oxysporium*

Wheat variety root exudate	Growth rate of Colony diameter (%) on day						
	2	3	4	5	6	7	7
L-18	136.36 ±8.86 ab	243.17 ±18.00bcd	384.73 ±16.07bc	420.22 ±22.04c	482.07 ±21.77c	543.57 ±26.09c	569.31 ±80.42c
L-17	136.54 ±26.62 ab	235.98 ±33.32cd	345.07 ±64.62c	433.16 ±53.72bc	495.99 ±63.02c	569.31 ±80.42c	492.82 ±35.11c
L-16	106.05 ±14.17 b	204.86 ±14.84d	292.98 ±24.85c	369.59 ±33.30c	435.59 ±37.63c	506.07 ±64.41c	565.38 ±61.31c
L-7	107.42 ±17.02 b	209.02 ±28.42d	335.78 ±26.51c	412.79 ±51.70c	506.07 ±64.41c	551.22 ±14.06bc	675.17 ±139.96bc
L-03K756	144.49 ±57.73ab	275.45 ±80.00abcd	372.74 ±42.61bc	493.46 ±113.85bc	551.22 ±14.06bc	675.17 ±139.96bc	837.36 ±164.72ab
D-05-4288	156.80 ±7.89ab	299.82 ±28.35abc	476.51 ±54.02ab	592.43 ±108.50ab	734.43 ±165.25ab	829.40 ±142.20ab	928.47 ±22.01a
D-08-8012	150.97 ±46.96ab	273.06 ±53.01abcd	459.98 ±83.24ab	589.77 ±104.92ab	733.91 ±123.01ab	829.40 ±142.20ab	928.47 ±22.01a
D-D	168.47 ±2.72a	324.67 ±11.77ab	525.46 ±22.89a	663.84 ±26.27a	819.74 ±31.31a	924.71 ±57.04a	965.49 ±135.58
Control	169.35 ±37.64a	326.90 ±49.14a	561.67 ±86.73a	707.80 ±116.35a	881.00 ±122.52a	965.49 ±135.58	-

Small letter indicated  $p < 0.05$  level. Different letters in the same column mean significant difference. Growth rate expressed in Percentage %. The concentration of extracts was 0.1 g/ml<sup>-1</sup>.

Table 2. Root exudates composition of 9-wheat varieties

Compound Number	Retention Time (min)	Peak Area (%)											
		Wheat varieties			Wheat varieties								
		L-18	L-17	L-16	L-7	L-03K76	D-05-4288	D-125	D-08-8012	D-D			
1	6.8	1.03	1.02	0.81	0.97	1.07	2.72	1.07	1.1	0.98			
2	6.91	0.53	0.49	0.42	0.44	0.46	1.19	0.53	0.53	0.59			
3	7.09	1.05	1.03	0.42	0.95	0.72	1.59	1.19	1.2	1.07			
4	7.28	0.37	0.39	1.03	0.39	0.72	1.38	0.45	0.44	0.55			
5	7.49	1.89	1.82	1.83	1.82	1.88	2.08	1.98	1.91	1.94			
6	7.66	0.13	0.08	0.12	0.13	0.09	0.33	0.12	0.11	0.12			
7	7.82	1.16	1.1	0.91	1.12	1.17	1.47	1.15	1.13	1.01			
8	7.95	3.43	4.3	3.48	4.38	4.2	6.1	3.84	5.18	3.62			
9	8.34	0.13	0.1	0.09	0.1	0.13	0.12	0.11	0.09	0.12			
10	8.62	-	-	-	-	-	-	-	-	-			
11	8.91	5.95	8.01	5.16	6.92	7.76	10.92	7.68	8.33	5.18			
12	17.09	0.17	0.16	0.17	0.15	0.14	0.5	0.14	0.52	0.14			
13	37.91	0.03	0.03	0.02	0.03	0.03	0.05	0.04	0.04	0.03			
14	8.19	13.74	12.95	13.78	13.28	12.98	11.31	13.53	12.23	13.77			
15	8.26	10.59	10.35	10.34	10.34	10.5	10.01	10.48	10.3	10.49			
16	9.18	46.38	44.43	47.19	46.34	44.68	37.03	45.06	44.07	47.61			
17	9.42	0.27	0.26	0.27	0.29	0.25	0.24	0.25	0.26	0.25			
18	11.15	10.31	10.31	10.54	9.23	10.35	8.66	9.47	9.57	9.6			
19	12.4	0.1	0.15	0.16	0.14	0.13	0.12	0.06	0.05	0.06			
20	13.99	0.4	0.39	0.39	0.39	0.38	0.38	0.38	0.38	0.38			
21	27.55	0.08	0.09	0.09	0.08	0.08	0.06	0.07	0.07	0.07			
22	33.61	0.06	0.04	0.15	0.07	0.03	-	-	-	-			
23	35.9	2.02	2.11	2.1	2.13	2.0	2.09	2.02	2.1	2.05			
Total		99.98	99.92	99.88	99.81	99.88	99.52	99.85	99.91	99.95			

“-”, not detected.

### Composition of root exudates of 9-wheat varieties

Root exudates of 9-wheat varieties were analysed using GC-MS (Table 2) and found to contain more than 30 organic components, of which 23 were very prominent. These were mainly hydrocarbons, alcohols, benzene, esters, ketones, aldehydes, diazole, organic acids and other substances. However, the esters were 77.83- 83.09% and the other compounds [including 1,2-Benzenedicarboxylic acid, Dibutyl phthalate and hexadecanoic acid] were about 20%. The composition of root exudates was not identical and the level of each component varied with the variety. Varieties L-18, L-17, L-16, L-7 and L-03K76 did not contain 4-Heptanol, 3-methyl- but varieties D-05-4288, D-125, D-08-8012 and D-D did not contain hexadecanoic acid.

Root exudates play major role in plant to plant communication and influences the rhizosphere microorganisms (13). Plant roots constantly secrete compounds into the soil to interact with neighboring organisms presumably to gain certain functional advantages, and the composition of root exudates varied at each developmental stage. This is genetically programmed (4). Plants exude a variety of substances through their roots, some of the compounds inhibits certain soil borne pathogens (1). The composition of root exudates from different varieties was different and had variable effects on the spore germination of *F. oxysporum* (2). Intercropping of aerobic rice with watermelon controls the *Fusarium* wilt disease in watermelon (19). Besides the aerobic rice roots release allelochemicals, which inhibits the growth of *F. oxysporum* and improves the soil environment for watermelon (19). The root exudates of marigold, rice, celery, and watermelon rootstock also inhibits the *F. oxysporum* hyphal growth (3,6,8,26).

Wheat (*Triticum aestivum* L.) is major cereal crop in world. Wheat, other cereals and wild gramineae species produce allelopathic compounds, these are present in wheat shoots and roots but roots are rich in these compounds (17,18,24). In this study, root exudates of wheat variety L-03K756, inhibited the spores germination and growth of *F. oxysporum*. Similar results were reported by Hu *et al* (10) and Wu *et al* (23). Further, the root exudates of different wheat varieties had variable affects on the fungus growth. Some varieties (L-18, L-17, L-16, L-7, L-03K756) significantly inhibited the growth of fungus after 4d, as they contained hexadecanoic acid and its content was maximum in variety L-16. The result showed that wheat cultivars have variable allelopathic potential, due to different allelochemicals present in them.

Preliminary analysis of wheat root exudates for various organic components by GC-MS showed that the exudates contain a variety of organic components [hydrocarbons, alcohols, benzene, esters, ketones, aldehydes, thiazole, organic acids]. Compounds secreted from the roots of various plants are different. The root exudates of cucumber, watermelon, tomatoes, peppers, eggplant and other vegetables contained various compounds [benzoic acid, cinnamon acid, coumaric acid, vanillic acid, ferulic acid, salicylic acid, dibutyl phthalate, phthalic acid, vanillin and palmitic acid (5,14,21,27,28,29,31)]. Of these, phthalate, Dibutyl phthalate and hexadecanoic acid allelochemicals are also present in root exudates of wheat. We did not investigate the key chemical compounds in 30 organic components, allelopathic to test pathogen. We did not investigate the key chemical compounds among the 30 organic components inhibitory to *F. oxysporum*. We studied only hexadecanoic acid for fungicidal activity. The hexadecanoic acid at 1 mmol·L<sup>-1</sup> inhibited the *F. oxysporum*. Zhou *et al* (30) also found that hexadecanoic acid reduced the number of soil fungi in eggplant rhizosphere. Its

proportion among the rhizosphere microorganisms increases the number of bacteria and actinomycetes ratio and stimulates the plant height and stem diameter of eggplant. The wheat varieties D-05-4288, D-125, D-08-0812 and D-D, which did not contain hexadecanoic acid, least inhibited the fungal growth (Table 1 and 2). Other compounds found in the root exudates need further study to explore their role in fungal growth inhibition. Some varieties especially variety L-16 strongly inhibited the growth of *F. oxysporum*, hence, it may be used in crop rotation or intercropping. Hexadecanoic acid accumulates in certain wheat varieties to act as a fungicidal i.e. as defence inducer. This possible additional advantages of root exudates allelochemicals is under study and opens an interesting field of research.

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