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Allelopathic effects of acetone extract from celery rotten roots and rhizosphere soil on cucumber pathogen *Fusarium oxysporum* f. sp. *cucumerinum*

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ABSTRACT

We studied the inhibitory effects of allelochemicals extracted from celery (*Apium graveolens* L.) rotten roots and rhizosphere soil on *Fusarium oxysporum* f. sp. *cucumerinum* (FOC). The allelochemicals were extracted and separated by column chromatography from the acetone extracts of celery rotten roots and rhizosphere soil. After extraction each fraction was mixed with potato dextrose agar (PDA) culture medium and then inoculated with FOC. The best fraction was screened by measuring the colony diameter and its effects on FOC was determined by evaluating the activities of antioxidant enzymes. The allelochemicals of best fractions were detected using GC-MS. The best fractions obtained after second run in column chromatography were labeled as RRA32, RRA38, RRA101, RRA102 and RRSA55, RRSA56, RRSA105, RRSA106 and they had allelopathic potential of 29.68 %, 31.97 %, 40.38 %, 41.55 % and 29.51 %, 29.47 %, 29.30 % and 32.85 % respectively. The antioxidants enzymes (peroxidase, catalase and superoxide dismutase) activities were significantly lower in treated FOC than control. Using the 8-best fractions, the GC-MS analysis yielded, total 47 compounds viz., 7 organic acids, 17 esters, 1 phenol, 1 alcohol, 1 aldehyde, 5 nitrogen-containing compounds and 2 carbides.

Keywords: Allelochemicals, allelopathy, antioxidant enzymes, celery, Column chromatography, *Fusarium oxysporum* f. sp. *cucumerinum*, GCMS, rotten roots and rhizosphere soil

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is important vegetable crop worldwide. In China, it is planted on 1.2 m ha in 2017 (25). Cucumber n is often grown in continuous monocropping systems due to limited arable land and ever-growing demand for it (37). The continuous monocropping of cucumber creates highly favourable conditions for soil pathogens, which significantly reduces its yield and quality (22,27), increased the root autotoxicity and decreases the root activity. The level of plant resistance to stress decreases and the plant becomes more vulnerable to soil-borne diseases (26). Cucumber *Fusarium* wilt is a soil-borne disease, caused *by Fusarium oxysporum* f. sp. *cucumerinum.* (FOC), in young and mature plants and is believed to be the major causal factor for the soil sickness in cucumber (2,7,18,33). Symptoms of the disease include wilting and eventual death (28). Fusarium can live in the soil as wilting chlamydospores for many years under various environmental conditions, hence, difficult to control this disease (1). In the past two decades, the most common method to control *Fusarium* wilt was grafting, prevention and use to fungicides. However, owing to the difficulty in grafting methods, complicated management after grafting and recent concerns form fungicides use, these are little used (23). Hence, the researchers have gradually paid more attention to allelopathy.

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Allelopathy is the effects of one plant or microorganism on the metabolic activities of other plants or microorganisms through release of chemicals in the environment (36). Allelopathy works through release of chemicals in the environment, which degrades easily to protect the environment and conform to the concept of modern green agriculture. The core of allelopathy is allelochemicals (21). The concentration of useful substances in allelochemicals is relatively low (12), there is difficulty in their separation, identification and purification. Now the allelochemicals are identified by gas chromatography mass spectrophotometer (GC−MS) and nuclear magnetic resonance (NMR) (29).

Figure 1. Celery crop grown in the field

Celery (*Apium graveolens* L) is vegetable crop with aromatic properties (Fig. 1) and grown worldwide due to high yields and economic benefits. However, only its above ground parts are used and underground parts are wasted. Our research team has (Figure 1), found that celery volatiles, seeds and extract of fresh roots and rhizosphere soil have allelopathic effects on FOC (3,6,16). The sixth chromatographic separation of fresh celery roots and rhizosphere soil have significant inhibitory allelopathic effects on FOC (10,31,32). The extracts of rotten celery roots and rhizosphere soil are inhibitory to FOC (4). However, the allelopathic effects of acetone extract from celery rotten roots and rhizosphere soil on FOC were not reported. This study aimed (i) to evaluate the allelopathic potential of the second time chromatographic separation of acetone extracts of celery rotten roots and rhizosphere soil and their role in controlling FOC and (ii) to chromatographically separate and identify the FOC inhibitory substances from celery rotten roots and rhizosphere soil and (iii) to provide a theoretical basis to prevent and control FOC. The best fraction was determined on the basis of toxicity level, and composition of best fraction was estimated using GC-MS.

MATERIALS AND METHODS

Materials

The celery cultivar ‘American celery’ seeds were purchased from the Seed Department, Inner Mongolia Academy of Agricultural Sciences. The pathogenic fungus

FOC was obtained from the Plant Pathology Department, Vegetable and Flower Research Institute, Chinese Academy of Agricultural Sciences, Beijing. Fungus was inoculated on a sterilized PDA culture medium (200 g of potato, 20 g of dextrose, 16 g of agar, 1000 mL of distilled water, and neutral pH) (0.1 MPa, 121 ºC, 30 min), cultivated for 7 days at 25 ºC and then stored in 4 ºC refrigerator for later use.

Collection of Celery Rotten Roots and Rhizosphere Soil

Celery seedlings were planted on August 7, 2018 in greenhouse, Inner Mongolia Agricultural University, Hohhot 010010, Inner Mongolia, China (40º 81 '65.16"N, 111º71'75.74"E). Total annual precipitation: 580.8 mm, Minimum and maximum temp: -24 ºC and 33 ºC in 2018. The above ground parts of mature celery plants were harvested on October 28, 2018 and the underground parts (roots) were left to decompose naturally in the soil. These rotten roots were collect on April 26, 2019 i.e. after 6-months in dry and loose state. Randomly collected 50 rotten roots of celery that were still in the original shape, the soil on the surface of these rotten roots were gently cleaned with brush and collected as 50 g rhizosphere soil. Then all the soil samples were mixed to make the homogenized composite sample. The rotten roots and rhizosphere soil of celery were separately packed in plastic bags, brought to the laboratory and placed at 4 ºC in refrigerator for later use.

Fifty g rotten roots and 50 g of rhizosphere soil were placed in separate flasks, mixed with 80 % acetone in 1:4 ratio and shaken for extraction for 24 h at 25 ºC, 240 rpm. The extract was filtered through 6-layers of gauze, and then through 5-layers of filter paper to obtain clear extract, called mother liquor. It was placed in refrigerator at 4 ºC for standby, if more was needed.

Chromatographic Separation of Extracts

Column chromatography was used to separate the components from acetone extracts of rotten roots and rhizosphere soil. As per the method of Yebing *et al*. (8), the chromatography column 100 mm × 300 mm was selected, silica gel of 100-200 mesh was used as stationary phase, mixed with 80 % acetone for wet column loading. 10 mL of clarified rotten roots extract and 10 mL of rhizosphere soil extract were respectively poured into the different silica gel column for chromatography, and 80 % acetone used as eluent. Five mL of each fraction was collected and 10 fractions were collected and labeled respectively (Rotten root: RRA1 to RRA10; Rotten root rhizosphere soil: RRSA1 to RRSA10).

Determining Allelopathy of Fraction and Selection of Best Fractions

The 10-fractions (RRA1 to RRA10) were obtained from celery rotten roots extract by column chromatography. Under aseptic conditions, these 10 fractions were respectively filtered through 0.45 μm bacterial filter, and then mixed with PDA culture medium in 1:9 ratio and poured into the plates. There were two controls, (i). 'CK' was mixed sterile water and PDA culture medium in 1:9 ratio and (ii). 'ACK', 80 % acetone and PDA culture medium was mixed in in 1:9 ratio. Each culture plates was added 2 mL of fractions and 18 mL of PDA culture medium. The treatments were replicates 5-times in Completely Randomised design. After the culture medium got solidified, FOC bacterial cake (6 mm dia) was placed in the centre of each plate and the plates were incubated at 25 ºC in dark for 7 days. After inoculating the FOC for 24 h, the colony size of each bacterial plate was measured by cross method and used to determine the allelopathic potential of each fraction.

Similarly, the 10 fractions (RRSA1 to RRSA10) were obtained from celery rotten rhizosphere soil extract by column chromatography. Under aseptic conditions, each fraction was filtered through a 0.22 μm bacterial filter respectively and then the allelopathy detection step was the same as that of celery rotten roots.

The allelopathic effect of extracts on FOC was calculated as under:

$$\text{Inhibitory effect (\%)} = \frac{\text{Control colony mean dia} - \text{Experimental colony mean dia}}{\text{Control colony mean dia}} \times 100$$

The best fractions obtained from the first time column chromatography of celery rotten roots and rhizosphere soil extracts were respectively loaded again in column chromatograph for second cycle and again 2- best fractions were screened out respectively.

Antioxidant enzymes in FOC Treated with Best Fraction

The best fractions identified in the celery rotten roots and rhizosphere soil extracts were filtered with bacterial filter, mixed with PDA culture medium in the proportion of 1:9 and poured into the plates, respectively. Acetone in ACK and sterile water in CK were added to equal amount of PDA culture medium. Each plate with 20 mL of mixed PDA culture medium, inoculated with FOC were incubated at 25 ºC for 7 days in dark, and each treatment was replicated five times in Completely Randomised design (39).

After 7 days of culture, 5 pieces of cake at the same position were punched from each treated bacteria plate, and placed in precooled mortar. Phosphate buffer (pH=7) 5 ml was added to the mortar and cakes were homogenised, then centrifuged at 4 ºC at 12,000 rpm for 15 min. The supernatant was treated as enzyme extract and used to determine the peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) enzymes.

(i). Peroxidase (POD) activity: It was measured by the guaiacol method (11). 0.2 mL of enzyme solution was added to 3 mL of mixed solution, the absorbance at 470 nm was recorded thrice at 1 min intervals.

(ii). Catalase (CAT) activity: It was determined according to H_2O_2 method (11). Initially 0.2 mL of enzyme solution (there were two control tubes, with equal amounts of phosphate buffer and boiled enzyme solution respectively), 1.5 mL of phosphate buffer and 1mL of distilled water were taken into the test tube, kept at 25 ºC temperature for 3 min. After cooling, 2.5 mL H_2O_2 was added, immediately recorded the absorbance at 240 nm 5- times, at 1 min intervals.

(iii). Superoxide dismutase (SOD) activity: It was measured by the NBT method (11). Taking 0.05 mL of enzyme solution (taking two test tubes added 0.05 mL of phosphate buffer solution, light control and dark control, respectively), 2.65 mL of reaction solution was added, shaking well and finally added 0.3 mL of riboflavin. Each treatment tube and the light control reacted under 2000 lux light intensity for 10 min (the dark control reacted in the dark place). Then absorbance was recorded at 560 nm.

Identification the Allelochemicals of Best Fraction

The best fraction screened from the second time chromatography was sent to the Key laboratory, Chinese Academy of Inspection for GC-MS (Finnigan TRACE DSQ GC-MS, United States). The specification of capillary column (DB-5) was: 30 m × 0.25 mm × 0.25 μm. The chromatographic conditions were as under: the initial temperature was 80 ºC (maintained for 5 min), and then it rises to 280 ºC at a speed of 15 ºC ·min^{-1} for 20 min. The carrier gas was helium (1 mL·min^{-1}), the injector temperature was 220 ºC, and the interface temperature was 250 ºC; Ionization mode EI, electron energy 70 eV, ion source voltage 220 V, detector voltage 350V; the injection volume was 1μl. The composition, name and content of allelochemicals in the best fractions were determined by manual analysis and check with the standard chromatogram.

Statistical Analysis

All data were processed by Microsoft Excel 2010 and analyzed by SAS9.0 for variance. Duncan multiple range test was done for the best fraction of each chromatography.

RESULTS AND DISCUSSION

Effects of Acetone extracts from celery rotten roots and rhizosphere soil

I. First time column chromatography fractions

(i). Celery rotten roots: The first time column chromatography fractions were inhibitory to FOC (Fig. 2). All 10-fractions (RRA1 to RRA10) obtained from the first time column chromatography of celery rotten roots extract were inhibitory to FOC. The RRA3 and RRA10 fractions were most inhibitory to FOC as evident from the significantly smaller colony diameters.

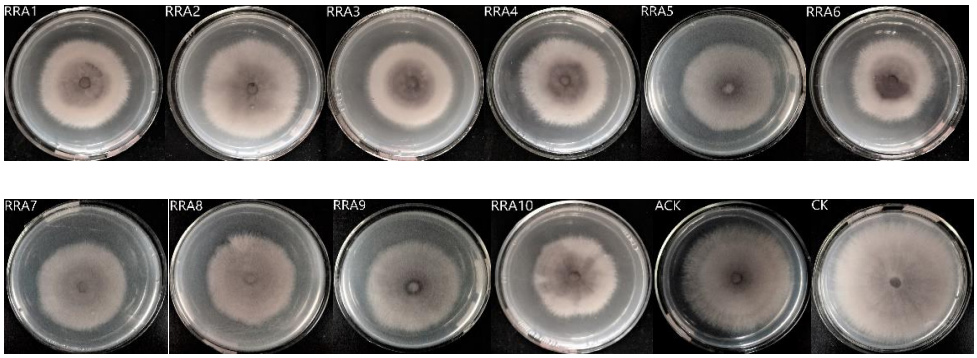

Figure 2. The effects of fractions from the first time chromatography of acetone extract of celery rotten roots on the colony diameter of *Fusarium oxysporum* f. sp. *cucumerinum* (FOC). RRA: the acetone extracts from celery rotten roots.

The 10-fractions obtained from the first time column chromatography were significantly more inhibitory than CK (blank control) and ACK (acetone control) (Fig. 3). Among them RRA3 and RRA10 were extremely inhibitory compared to controls. The allelopathic effects of RRA3 and RRA10 were 23.11 % and 24.34 % and 18.64 % and 19.93 % higher over CK and ACK, respectively. Thus, these two fractions RRA3 and RRA10 most inhibitory to FOC were subjected to second time column chromatography for further separation of fractions.

(ii). Celery rhizosphere soil: Ten fractions (RRSA1 to RRSA10) obtained by column chromatography from celery rotten rhizosphere soil extract were allelopathically inhibitory to FOC (Fig. 4). The colony diameters of fractions RRSA5 and RRSA10 were smaller than CK, ACK and other fractions.

The allelopathy of 10-fractions obtained from the first time column chromatography of celery rotten rhizosphere soil were significantly more inhibitory than CK (blank control) and ACK (acetone control) (Fig. 5). Among all fractions, RRSA5 and RRSA10 were allelopathically most inhibitory, causing inhibition of 30.9 % and 21.14 %, and 27.87 % and 17.66 % over CK and ACK, respectively. Consequently, these two fractions were subjected to the second time column chromatography for further separation of fractions.

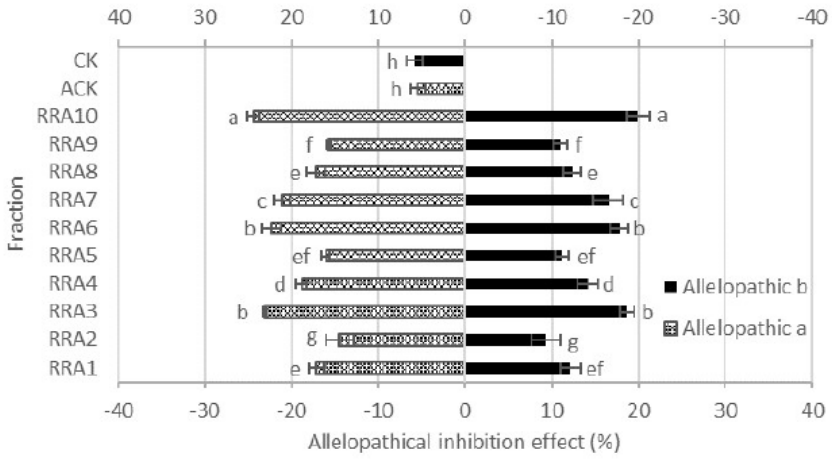

Figure 3. Allelopathic effect of fractions from first time column chromatography of acetone extract of celery rotten roots on FOC. RRA: the acetone extracts from celery rotten roots. Allelopathic a: the allelopathic effects compared with CK (blank control). Allelopathic b: the allelopathic effects compared with ACK (acetone control). Values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test)

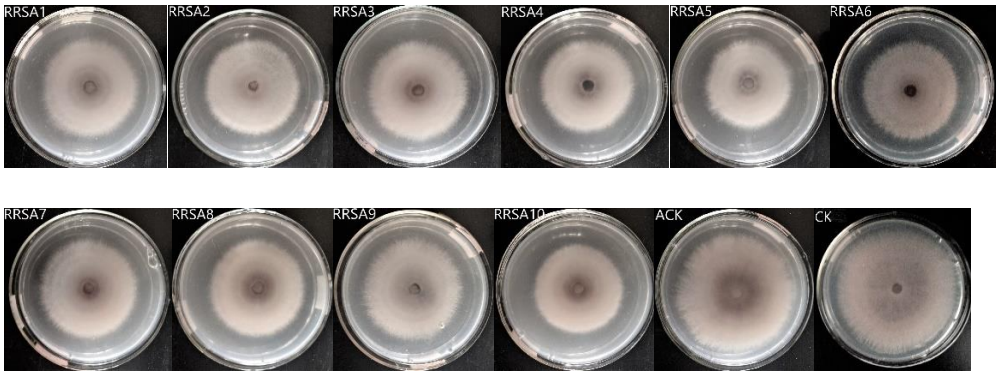

Figure 4. The effects of fractions from the first time chromatography of acetone extract of celery rotten rhizosphere soil on the colony diameter of FOC. RRSA: the acetone extracts from celery rotten rhizosphere soil.

II. Second time Column Chromatography

(i). Celery rotten roots: The two best fractions RRA3 and RRA10 of celery rotten roots extract determined by the first time column chromatography were subjected to the second time column chromatography. Two best allelopathic fractions were also obtained from the second time column chromatography and finally we obtained 4-best fractions of celery rotten roots extract.

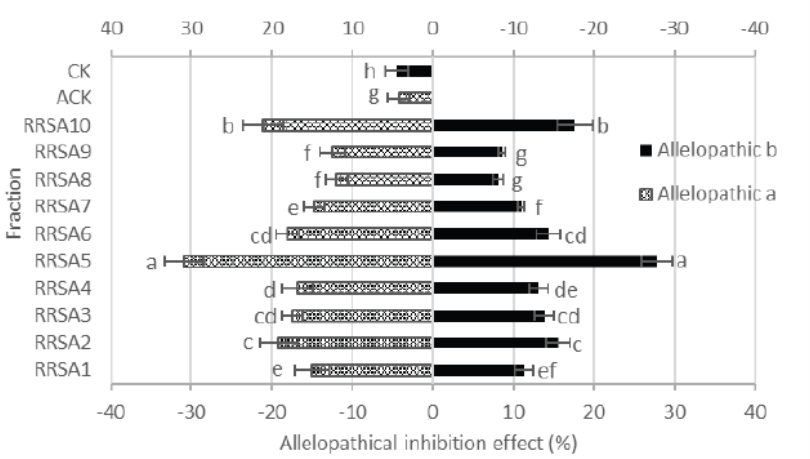

Figure 5. Allelopathic effects of fractions obtained from the first time column chromatography of acetone extract of celery rotten rhizosphere soil on FOC. RRSA: the acetone extracts from celery rotten rhizosphere soil. Allelopathic a: the allelopathy effects compared with CK (blank control). Allelopathic b: the allelopathy effects compared with ACK (acetone control). Values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test.

(ii). Celery rhizosphere soil: The same screening was done on the celery rotten rhizosphere soil extract. Total 8-best fractions were obtained from the two extracts.

The eight best fractions of the second time column chromatography had allelopathic inhibitory effects on FOC, and the colony diameter was drastically decreased than respective control (Fig. 6). RRA102 ("10" represents the best fraction obtained by the first time column chromatography of the acetone extract of celery rotten roots, "2" represents the best fraction selected by the second time chromatography of "10" fractions. So on for other best fractions) has the smallest colony diameter reflecting the strongest inhibitory effect on FOC. The comparison between with the two extracts, showed that the allelopathy of celery rotten roots was stronger than celery rotten rhizosphere soil.

The allelopathic inhibitory effects of 8-best fractions were significant by inhibitory over the control (Fig. 7). Among them, RRSA106 was most inhibitory than control in RRSA series, causing 32.86 % and 30.3 % inhibition compared to CK and ACK, respectively. In the 4 fractions of rotten celery roots, the allelopathic effects of RRA101 and RRA102 were 40.38 % and 41.55 % and 38.11 % and 39.33 % higher over CK and ACK, respectively. Among the other two fractions, the RRA38 was significantly higher than RRA32.

Allelopathy works mainly through allelochemicals, which are released through volatilization, leaching from leaves, degradation of plant residues and root exudation (5). Zhang (38) studied the allelopathic effects of the pure water and 75 % ethanol extracts of *Kandelia candel* on *Phytophthora infestans*. The results showed that the 75 % ethanol and pure water extracts of *K. candel* were allelopathically inhibitory to *P. infestans*. Wang (34) studied the allelopathic potential of root exudates of *Vicia faba*, corn and garlic on root rot pathogens of *Panax notoginseng*. The root exudates of these 3- plants had inhibitory effects on root rot pathogens of *P. notoginseng*. Our research group had done much research on allelopathy. The *Fusarium oxysporum* of cucumber was treated with extracts of fresh celery roots and rhizosphere soil the results demonstrated that the allelopathic properties of celery roots substances were inhibitory to *F. oxysporum* of cucumber at high concentrations. In

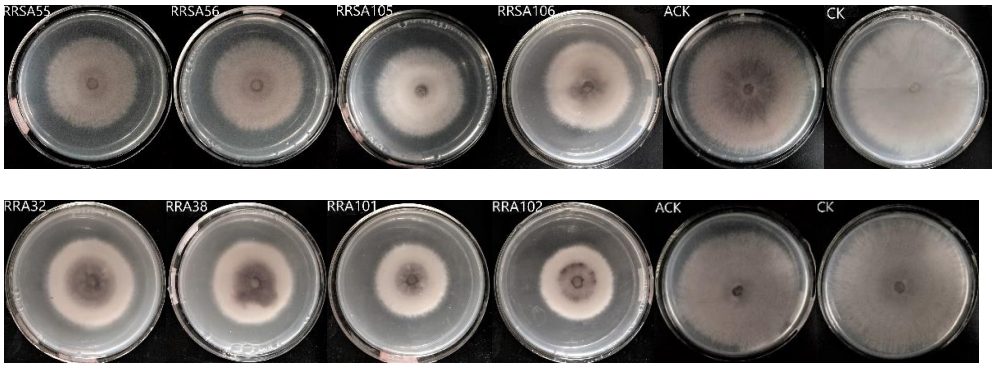

Figure 6. The effects of best fractions from the second time chromatography of acetone extracts of celery rotten roots and rotten rhizosphere soil on the colony diameter of FOC. RRA: the acetone extracts from celery rotten roots. RRSA: the acetone extracts from celery rotten rhizosphere soil.

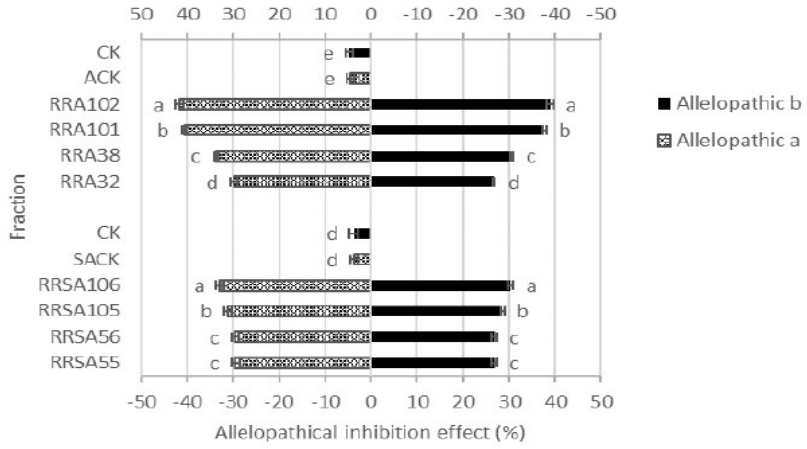

Figure 7. Allelopathic effects of best fractions obtained from the second time column chromatography of acetone extracts of celery rotten roots and rotten rhizosphere soil on FOC. RRA: the acetone extracts from celery rotten roots. RRSA: the acetone extracts from celery rotten rhizosphere soil. Allelopathic a: Allelopathic effects compared with CK (blank control). Allelopathic b: Allelopathy effects compared with ACK (acetone control). Different letters indicate significant differences at $P < 0.05$ according to Duncan's multiple comparison test.

addition, Chen (6) proved that celery roots substances and volatiles had negative effects on *F. oxysporum* of cucumber. The diameter of *F. oxysporum* colony decreased significantly by the fresh roots and rhizosphere soil extracts of celery, obtained after four time run through chromatograph (10). Sun and Li, Su and Qin (31,33) extracted fresh roots and rhizosphere soil of celery with ethanol and acetone, the extracts were chromatographed 6- times and the results showed that allelopathy was further strengthened. In this study, treated FOC with fractions from twice chromatography of acetone extracts of celery rotten roots and rhizosphere soil, the results showed that the best fractions had significant allelopathic

inhibitory effects on FOC, similar to previous research results. With the increase of chromatography times of extract, the colony diameter was significantly smaller than the control. This was because the allelochemicals were further separated and purified by chromatography, which laid the foundation for obtaining more specific substances later.

Effects of Best Fraction on Antioxidant System Enzymes

The best fractions obtained from second time column chromatography of acetone extract of celery rotten roots and rhizosphere soil decreased the activities of antioxidant enzymes (POD, CAT and SOD) in FOC than control (Fig. 8, 9 and 10). In RRA series, the activities of POD and CAT differed significantly among the RRA32, RRA38, RRA101 and RRA102, the SOD activities of RRA101 and RRA102 were significantly higher than RRA32 and RRA38. In RRSA series, the POD and SOD activities of RRSA105 and 106 were significantly higher than RRSA55 and 56, while the differences in CAT activities were non-significant.

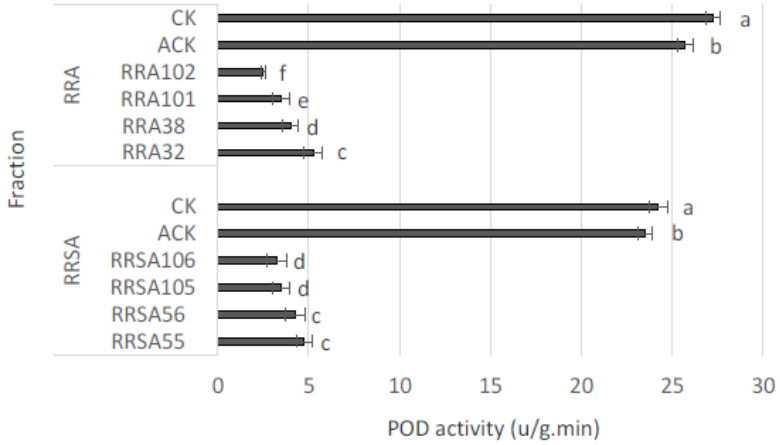

Figure 8. Effects of best fractions treatment of second time chromatography of RRA and RRSA series on POD Activity. RRA: the acetone extracts from celery rotten roots. RRSA: the acetone extracts from celery rotten rhizosphere soil.

Many studies have shown that allelochemicals significantly inhibits the activities of antioxidant enzymes and increase the level of free radicals (9). Jia (17) studied the changes of POD, CAT and SOD activities of FOC treated with the extract of celery seed. The results showed that the activity of antioxidant enzymes was lower than control; Song and Gao (11,31) studied the allelopathic mechanism of the extract of celery fresh roots on FOC. The results were similar to Jia (17). After treatment, the POD, CAT and SOD activities in FOC were lower than in control. Zhang (39) studied the allelopathic mechanism of *C. coronarium* crude extract on watermelon *Fusarium* wilt. The results showed that after being treated with different concentrations of *C. coronarium* crude extract, the SOD and CAT enzyme activities decreased than control in watermelon *Fusarium* wilt pathogen. With the increase of crude extract concentration, enzyme activity gradually decreased. In this experiment, after FOC was treated with the best fractions from second time chromatography of acetone extracts of celery rotten roots and rhizosphere soil, the activities of (POD, CAT and SOD) in the bacteria were extremely lower than control, which was similar to our previous research results. In the antioxidant system, POD, CAT and SOD are protective enzymes. Higher the activity level of antioxidant enzymes, greater is the resistance to external hazard. In this

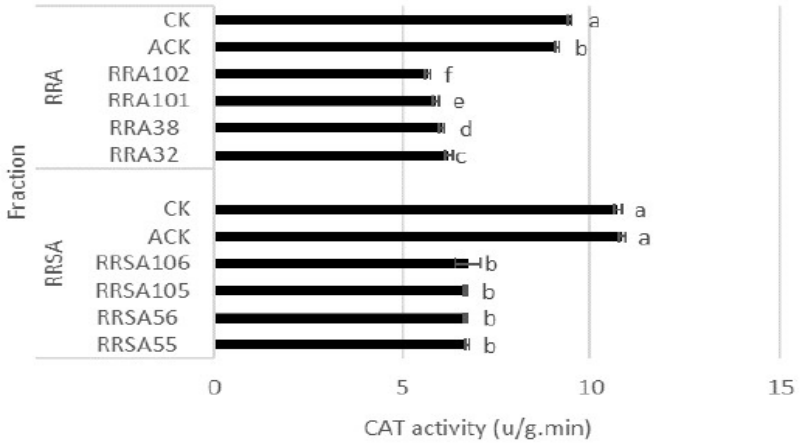

Figure 9. Effects of best fractions treatment of second time chromatography of RRA and RRSA series on CAT Activity. RRA: the acetone extracts from celery rotten roots. RRSA: the acetone extracts from celery rotten rhizosphere soil.

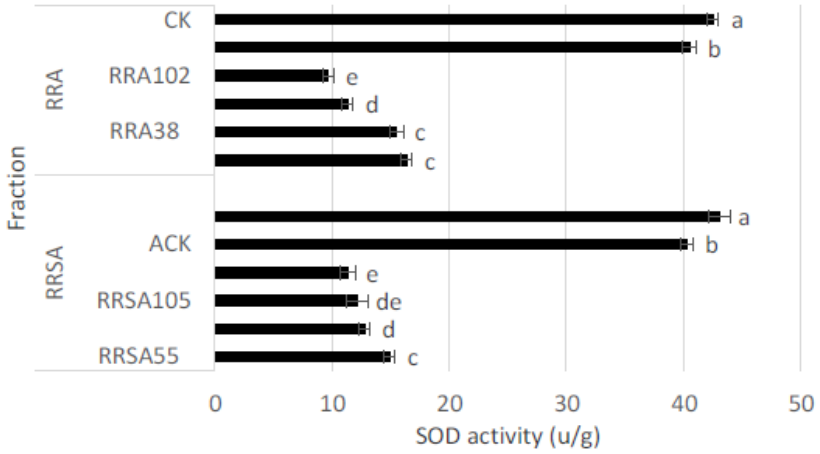

Figure 10. Effects of best fractions treatment of second time chromatography of RRA and RRSA series on SOD Activity. RRA: the acetone extracts from celery rotten roots. RRSA: the acetone extracts from celery rotten rhizosphere soil.

study after FOC was treated, the activity of antioxidant enzymes decreased, indicating that their ability to resist the external environment was decreased in the bacteria, and thereby the allelopathy inhibited the growth of FOC.

Identification of Allelochemicals

After the second time chromatography, the GCMS chemical analysis of 8-best fractions of celery rotten roots and rhizosphere soil yielded peaks as under: RRA32 has 22 peaks, RRA38 has 19 peaks, RRA101 and RRSA55 have 18 peaks, RRA102 has 17 peaks, RRSA56 has 11 peaks, RRSA105 has 14 peaks and RRSA106 has 15 peaks. The allelochemicals identified by GCMS analysis included 7 organic acids, 17 esters, 1 phenol, 1 alcohol, 1 aldehyde, 5 nitrogen compounds and 2 carbides (Table 1).

Table 1. GC-MS analysis results of specific peaks of best fractions for second time chromatography of acetone extracts from celery rotten root and rhizosphere soil

Best fraction	Retention time	Name of compound	Molecular formula	Molecular weight
RRA32	10.499	Tricosanoic acid	$C_{23}H_{46}O_2$	354
	11.744	2-Dihydro-2-naphthalenyl acetate	$C_{12}H_{12}O_2$	188
	12.783	Methyl 4-(2,5-dimethylphenyl)-3-methylbutanoate	$C_{14}H_{20}O_2$	220
	13.139	Tetradecane	$C_{14}H_{30}$	198
	13.302	3-Isopropylbenzaldehyde	$C_{10}H_{12}O$	148
	13.683	2-Amino-3-naphthalen-2-ylpropionic acid	$C_{13}H_{13}NO_2$	215
	13.952	2-Naphthaleneacetic acid, ethyl ester	$C_{14}H_{14}O_2$	214
	15.241	5-Isopropyl-2-methylphenethyl acetate	$C_{14}H_{20}O_2$	220
	15.579	Methyl4-methylene-2-phenylcyclopentanec arboxylate	$C_{14}H_{16}O_2$	216
	15.754	1-Naphthalenemethanamine	$C_{11}H_{11}N$	157
RRA38	4.845	Tert-Butyl Acetate	$C_6H_{12}O_2$	116
	13.69	2-Amino-3-naphthalen-2-ylpropionic acid	$C_{13}H_{13}NO_2$	215
	13.952	1-Naphthaleneacetamide	$C_{12}H_{11}NO$	185
	16.636	Ethyl 3-ethoxypropionate	$C_7H_{14}O_3$	146
	16.992	Ethyl 4-t-butylbenzoate	$C_{13}H_{18}O_2$	206
	17.418	2,2-Diphenylacetamide	$C_{14}H_{13}NO$	211
RRA101	4.838	Dimethyl malonate	$C_5H_8O_4$	132
	13.308	2-Allyl-4-methylphenol	$C_{10}H_{12}O$	148
	13.69	3-(2-Naphthyl)alanine	$C_{13}H_{13}NO_2$	215
	13.959	1-(1H-Inden-1-yl)ethyl acetate	$C_{13}H_{14}O_2$	202
	15.766	1-Naphthalenemethanamine	$C_{11}H_{11}N$	157
	16.636	Ethyl 3-ethoxypropionate	$C_7H_{14}O_3$	146
	16.986	4'-Diethylaminoacetanilide	$C_{12}H_{18}N_2O$	206
	18.775	2,2-Diphenylpropionic acid	$C_{15}H_{14}O_2$	226
RRA102	4.876	Isobutyldimethylcarbinol	$C_7H_{16}O$	116
	13.69	2-Amino-3-naphthalen-2-ylpropionic acid	$C_{13}H_{13}NO_2$	202
	13.946	1-Naphthaleneacetamide	$C_{12}H_{11}NO$	185
	15.572	1-Phenyl-3-methylpenta-1,2,4-triene	$C_{12}H_{12}$	156
	16.285	Protoporphyrin IX	$C_{34}H_{34}N_4O_4$	562
	16.986	3,4-Dimethyl-2-(3-methyl-butyryl)-benzoic acid, methyl ester	$C_{15}H_{20}O_3$	248
RRSA55	13.946	Methyl 1-naphthaleneacetate	$C_{13}H_{12}O_2$	200
	16.673	Ethyl 3-hydroxypentanoate	$C_7H_{14}O_3$	146
RRSA56	10.493	Tricosanoic acid	$C_{23}H_{46}O_2$	354
	12.789	3,5-Dimethylbenzyl 2,4-dimethylbenzoate	$C_{18}H_{20}O_2$	268
	13.702	Methyl 1-naphthaleneacetate	$C_{13}H_{12}O_2$	200
	13.99	1-Naphthaleneacetamide	$C_{12}H_{11}NO$	185
	16.993	Methyl p-tert-butylphenylacetate	$C_{13}H_{18}O_2$	206
	24.217	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
RRSA105	4.863	Tert-Butyl Acetate	$C_6H_{12}O_2$	116
	14.021	Hexyl 1-naphthylacetate	$C_{18}H_{22}O_2$	270
	15.672	Decanoic acid	$C_{10}H_{20}O_2$	172
	17.005	Ethyl 4-t-butylbenzoate	$C_{13}H_{18}O_2$	206
RRSA106	10.481	Tricosanoic acid	$C_{23}H_{46}O_2$	354
	13.733	1-Naphthaleneacetic acid	$C_{12}H_{10}O_2$	186
	13.965	2-Amino-3-naphthalen-2-ylpropionic acid	$C_{13}H_{13}NO_2$	202
	16.992	Methyl p-tert-butylphenylacetate	$C_{13}H_{18}O_2$	206

RRA: the acetone extracts from celery rotten roots. RRSA: the acetone extracts from celery rotten rhizosphere soil.

In the identification and allelopathy of green garlic volatiles on scavenging of cucumber reactive oxygen species (ROS) (24), the allelochemicals of garlic were detected by GC-MS. In this study, GC-MS was used to detect the constituents of the best fraction, and in the end, 7- organic acids were obtained, (2,2-Diphenylpropionic acid, 3-(2-Naphthyl)alanine, 1-Naphthaleneacetic acid, 2-Amino-3-naphthalen-2-ylpropionic acid, Tricosanoic acid, Decanoic acid and n-Hexadecanoic acid). 17 kinds of esters [Ethyl 3-ethoxypropionate, tert-Butyl Acetate,1-(1H-Inden-1-yl)ethyl acetate, Methyl p-tert-butyl phenyl acetate, Methyl 4-(2,5-dimethylphenyl)-3-methyl butanoate, 2-Naphthaleneacetic acid, ethyl ester, 5-Isopropyl-2-methylphenethyl acetate,2-Dihydro-2-naphthalenyl acetate, Ethyl 4-t-butylbenzoate, Hexyl 1-naphthylacetate, Ethyl 3-hydroxypentanoate, Methyl 1-naphthaleneacetate, 3,5-Dimethylbenzyl 2,4-dimethylbenzoate, 3,4-Dimethyl-2-(3-methyl-butyryl)-benzoic acid, methyl ester, Dimethyl malonate and Methyl 4-methylene-2-phenylcyclopentanecarboxylate] were identified. Phenolic acids included 2-Allyl-4-methyl phenol. Alcohols were: Isobutyl dimethyl carbinol. Aldehyde included 3-isopropyl benzaldehyde. Nitrogen-containing compounds (1-Naphthalene methanamine, 1-Naphthalene acetamide, IX Protoporphyrin IX, 2,2-Diphenyl acetamide and 4'-Diethylamino acetanilide) were also detected. Carbide identified in the extracts included 1-Phenyl-3-methylpenta-1,2,4-triene and Tetradecane. The detailed study of GC-MS data revealed that esters were the most dominant group followed by organic acids and lastly nitriles.

The allelochemicals mainly include esters (35), alcohols, phenols, organic acids and derivatives, etc (14). Because this study was done with twice chromatography of extracts, more allelochemicals were detected, with 8 fractions and a total of 47 compounds. Previous studies have shown that diisobutyladipate (41) had negative effects on *Fusarium oxysporum* f. sp. *vasinfectum*, while benzyl benzoate (40) had significant inhibitory effects on eggplant *Verticillium* wilt. Esters accounted for the largest proportion of allelochemicals detected in this study, and were the main allelochemicals. Combined with this study, esters the main allelochemicals, could lay foundation for future research on biological agents. There had been many studies on organic acids as allelochemicals (19). Previous studies have proved that the allelopathy of wheat was related to the existence of simple phenolic compounds, including [p-hydroxybenzoic acid, vanillic acid, syringic acid and ferulic acid (20)]. Hou (15) studied the allelopathy of *Ginkgo biloba* extract on Capsicum wilt and detected 22 allelochemicals, including 8 organic acids [2- hydroxypropionic acid, benzoic acid, myristic acid, 3- hydroxybenzoic acid, 3,4-dihydroxycinnamic acid, n-hexadecanoic acid, 8- octadecenoic acid and eicosanoic acid]. In this study, there was one phenol, 2- allyl -4- methylphenol and 7 organic acids [2,2- diphenylpropionic acid, 3-(2- naphthyl) alanine, 1- naphthylacetic acid, 2- amino -3- naphthalene -2- ylpropionic acid, tricosanoic acid, decanoic acid and n-hexadecanoic acid]. Among them, n-hexadecanoic acid wass also been detected indicating that it had allelopathic inhibitory effects on *Fusarium oxysporum* similar to that against Capsicum wilt and can become a key substance for future research. In the study of identification and allelopathy of green garlic volatiles on scavenging of cucumber (9), it was found that the long chain hydrocarbon volatile compounds may be impurities in air. In our study, hydrocarbon compounds were also detected, which proved that all detected substances were not allelochemicals. To obtain more specific allelochemicals, one should use column chromatography to separate and purify the extract many times, and further researches should be conducted with specific allelochemicals.

CONCLUSIONS

We compared the effects of fractions from 2- times column chromatography of acetone extracts of celery rotten roots and rhizosphere soil on colony diameter and allelopathic effects on FOC (*Fusarium oxysporum* f. sp. *cucumerinum*). We screened 8, best

fractions (RRA32, RRA38, RRA101, RRA102 and RRSA55, RRSA56, RRSA105, RRSA106) obtained from second tine column chromatography. Then determined the effects of these best fractions on the activities of POD, CAT and SOD in FOC, the enzymatic activities were significantly decreased than ACK and CK. By using GC-MS, a total of 47 compounds were obtained from best fractions, these were mainly esters, organic acids and nitrides. It showed that the best fractions from celery rotten roots and rhizosphere soil were allelopathic and reduced the antioxidant system enzymes activity of FOC, inhibited FOC growth and thereby, prevented the cucumber fusarium wilt.

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