Mycopathologia (2008) 166:93–102
DOI 10.1007/s11046-008-9124-1

Biological Control of Phytopathogenic Fungi by Fatty Acids

Siyun Liu · Weibin Ruan · Jing Li · Hua Xu · Jingan Wang · Yubao Gao · Jingguo Wang

Received: 18 March 2008 / Accepted: 16 April 2008 / Published online: 29 April 2008

Abstract The aim of the present study was to evaluate the antifungal activity of fatty acids against phytopathogenic fungi. Two pot experiments were conducted by mixing palmitic and oleic acids in the soil in which poor plant growth was observed. In addition, the antifungal activities of nine fatty acids (butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, oleic acid, and linoleic acid) against four phytopathogenic fungi: *Alternaria solani*, *Colletotrichum lagenarium*, *Fusarium oxysporum* f. sp. *Cucumerinum*, and *Fusarium oxysporum* f. sp. *lycopersici*, were assessed by measuring mycelial growth and spore germination via Petri dish assay. The results of the pot experiments showed that the mixture of palmitic and oleic acids enhanced the growth of the seedlings of continuous-tomato and continuous-cucumber. Except for oleic acid, in the Petri dish assay, the fatty acids tested were observed to inhibit the mycelial growth of one or more tested fungi. In addition to the suppression of mycelial growth, butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, and palmitic acid showed an inhibitory effect against spore germination and the extent of inhibition varied with both the type of fatty acids, and the fungi. In particular, capric acid displayed strong inhibitory effect against *C. lagenarium* on the mycelial growth and spore germination. The saturated fatty acids, i.e. palmitic acids, showed stronger antifungal activity than the unsaturated fatty acids, i.e. oleic acid. It suggests that fatty acids might be applicable to exploring for alternative approaches to integrated control of phytopathogens.

Keywords Fatty acids · Antifungal · Phytopathogenic fungi · Mycelial growth · Spore germination

S. Liu · W. Ruan (✉) · J. Li · H. Xu · Y. Gao
College of Life Sciences, Nankai University, Building 4, Room 303, Tianjin 300071, People's Republic of China
e-mail: ruanweibin2004@hotmail.com

S. Liu · J. Li · J. Wang
College of Chemistry and Biology, Tianjin Normal University, Tianjin 300074, China

J. Wang
College of Resources and Environmental Sciences, China Agricultural University, Beijing 100094, China

Introduction

Various seed cakes, the by-products of vegetable oil extraction containing fatty acids, have been traditionally applied to agricultural field owing to better food quality and higher resistance to plant pathogen attacks, although the mechanism remains unclear. Fatty acids (FAs), ubiquitous in nature, play a crucial role in life process. FAs belong to a physiologically important class of molecules involved in cell energy storage, membrane structure, and various signaling pathways. In addition, fatty acids in animal fat, plant

oil, and other sources are an important part of human nutrition. Researchers have found that a large number of fatty acids have antimicrobial activities and potential as medicine in nutritional therapy. Kabara et al. [1] demonstrated that lauric acid, oleic acid, and linoleic acid possessed biocidal effects against gram-positive bacteria. Moreover, many long-chain unsaturated fatty acids have been shown to have antimalarial effect due to its inhibition of the biosynthesis of fatty acids in the parasite *Plasmodium falciparum* which is responsible for the most severe and deadly form of malaria [2]. Recently, it has been demonstrated that 2-alkynoic fatty acids could inactivate the pathogen of tuberculosis such as *Mycobacterium tuberculosis* H37Rv via dual inhibition of biosynthesis and degradation of fatty acids, indicating some fatty acids have the potential in antituberculosis therapy [3]. Myristic acid, lauric acid, capric acid, palmitoleic acid, and oleic acid have an antimicrobial activity against the pathogens of tuberculosis [2]. At the same time, some fatty acids were also found to have antifungal activity. For instance, capric acid has been reported to possess the greatest cytotoxicity against *Candida albicans* [4], and (Z)-9-heptadecenoic acid has an inhibitory effect against the fungi *Phytophthora infestans* and *Idriella bolleyi* [5]. Although the potential of fatty acids in medical applications has been intensively investigated, the documents related to the role of fatty acids in plant pathogen control of agroecosystem were relatively rare. Only few studies have been documented in this area: lauric acid [6, 7] and some unsaturated fatty acids [8] have been shown to possess the inhibitory effect on several plant pathogens.

Many plant diseases can drastically reduce yield and quality of crops, and are major restraints in sustainable agriculture production, especially in the intensive cropping systems. A number of chemicals are applied to control plant diseases worldwide. Owing to the negative impact of chemicals on non-target organisms and the potential of environmental and health risk, the range and rate of pesticides used were gradually limited and some of them were phased out. For example, methyl bromide identified as Class I stratospheric ozone depleting substance, used as a pre-plant soil fumigant for over 40 years, is being phased out worldwide until 2015 [9]. Therefore, finding alternative approaches to control plant pathogens are urgent and relevant studies have increased rapidly. In our previous experiment, with a salt-affected soil (pH 8.00) collected from the greenhouse where cucumber plants were continuously cropped for 7 times within 4 years [10] and the symptoms of plant wilt and death was occasionally observed, we confirmed the enhance growth effect of sesame (*Sesamum indicum* L.) cake (5‰ w/w sesame cake crude extracts in soil) [10]. Thirty kilograms *Sesamum indicum* L. cakes were extracted by 95% ethanol for 8 h under reflux to obtain 4.5 kg of the crude extract. Subsequently, oleic acid and palmitic acid were separated and identified as the major active substances, by GC-MS in the CCl_4 fraction of sesame cake crude extracts, after several attempts for extraction with organic solvents. In sesame cake crude extract, the proportion of each of oleic acid and palmitic acid reaches to approximately 20% of the dry weight of crude extracts of sesame cake. It meant that the final concentration of pure palmitic acid and oleic acid in soil was 1‰ w/w each and a significant growth enhancement of continuous-cucumber was identical to the result obtained with the application of 5‰ sesame cake crude extracts [11].

Based on others' studies and our previous research results, we hypothesize that fatty acids might have great antifungal potential in controlling the soilborne plant pathogens and could be attributed to the growth enhancement of continuous-cucumber mentioned in the reference [10, 11]. Taking into consideration of worldwide consumer demand of fatty acids as safe food with environment friendly attributes, it is essential to evaluate the impact of fatty acids against soil-borne microbe and have intensive study on their applications in the sustainable agricultural production. The effects of fatty acids added into the soil on plant growth enhancement may vary greatly with soil types, plant species, fatty acids, and soil borne pathogens. Thus, in this study, another soil type—cinnamon soil was employed to evaluate the plant growth enhancement of palmitic acid and oleic acid.

The objectives of this study were: (1) to evaluate the combination of palmitic acid and oleic acid for their effects on the growth of continuous-tomato and continuous-cucumber grown in the cinnamon soil in greenhouse, and (2) to assess potential antifungal activities of nine fatty acids against four selected economically important plant-pathogenic fungi at different concentration levels.

Materials and Methods

Fatty Acids

The saturated fatty acids (FAs) tested for antifungal activity were butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0). The unsaturated fatty acids oleic acid (C18:1Δ^{9c}) and linoleic acid (C18:2$\Delta^{9c,12c}$) were also evaluated. All the reagents were purchased from Tianjin Reagent Corp, P. R. China.

Fungal Strains and Growth Conditions

Alternaria solani, *Fusarium oxysporum* f. sp. *Cucumerinum*, and *Fusarium oxysporum* f. sp. *lycopersici* were obtained from the Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University. *Colletotrichum lagenarium* was obtained from State Key Laboratory of Elemento-organic Chemistry, College of Chemistry, Nankai University.

The fungi were grown initially on potato dextrose agar (PDA; pH 6.50) in dark and the culture stocks were stored at 4°C. The plant-pathogenic fungi were routinely sub-cultured on PDA (pH 6.50) in Petri dishes (90 mm in diameter) in dark at 25°C so that the fresh culture can be prepared for later use.

Preparation of Spore Suspensions

Fusarium oxysporum f. sp. *cucumerinum* and *Fusarium oxysporum* f. sp. *Lycopersici* were cultured for approximately 7 days at 25°C on PDA (pH 6.50), which was the most suitable medium for promoting spores of the fungi. To produce abundant spores of *Colletotrichum lagenarium*, we used the medium consisting of: 300 g of potato tubers (boiled for 30 min then filtrated to obtain the extract), 15 g of sucrose, 2 g of peptone, 0.5 g of Na_2HPO_4, 0.5 g of $Ca(NO_3)_2$, and 15 g of agar in 1,000 ml distilled water. Spore suspensions were prepared by flooding the sporulating cultures with sterile distilled water, passing the spore suspension through three layers of cheese-clothes to remove mycelia. Spore concentrations were determined by a hemacytometer and diluted with sterile distilled water to a concentration of 5×10^5 spores/ml [12]. It was noted that spore suspensions were made immediately just before each individual experiment. Because it was hard for us to find the culture medium and conditions for spore production of *Alternaria solani*, the fungus was excluded in the test of FAs' inhibitory effect on their spore germination.

Pot Experiment

The pot experiment was conducted in the greenhouse of College of Life Sciences, Nankai University. Tomato (*Lycopersicon esculentum* cv. Fenguan1) seeds and cucumber (*Cucumis sativus* L. cv. Jinchun 4) seeds were kindly provided by Tianjin Academy of Agricultural Sciences. Soils were collected from the spots where visible poor growth of cucumber or tomato plants occurred due to continuous monoculture of cucumber or tomato in corresponding greenhouses (soil in continuous-cucumber greenhouse pH 7.00, in continuous-tomato greenhouse pH 6.03), Shouguang, Shandong Province, P. R. China. After air-drying at room temperature, 0.8 kg of soils were placed into each pot and then thoroughly mixed with fertilizers (0.74 g K_2SO_4 per kg dry soil, 0.60 g $CO(NH_2)_2$ per kg dry soil, 0.48 g $(NH_4)_2HPO_4$ per kg dry soil). After that, palmitic acid and oleic acid were added separately and thoroughly mixed, including a low level: 1‰ palmitic acid w/w (3.9 mmol palmitic acid per kg dry soil) and 1‰ oelic acid w/w (3.2 mmol oleic acid per kg dry soil) [11] and a high level: 2‰ palmitic acid w/w (7.8 mmol palmitic acid per kg dry soil) and 2‰ oleic acid w/w (6.4 mmol oleic acid per kg dry soil). The reason for only choice of combination of palmitic and oleic acids in the pot experiment was owing to their presence in large quantity in sesame cake and its application resulted in a better growth and higher resistance to pathogens [10, 11]. Pots without these two fatty acids were used as the control. Ten uniform seeds of cucumber or tomato were sown in the corresponding pot and emerged seedlings were thinned to three per pot and placed in the greenhouse. All the pots were watered as needed. Treatments were arranged in a completely randomized design with six pots per treatment. The cucumber and tomato plants were harvested 22 and 30 days after planting, respectively. The shoots and roots were oven-dried at 105°C for 30 min and then at 80°C for 48 h, and finally were weighed. Both experiments were conducted twice; the results were

similar, only the results of one of each experiment are shown.

Effects of Fatty Acids on Mycelial Growth of Pathogens

Based on the results obtained in the pot experiment, effects of palmitic and oleic acids on both mycelial growth and spore germination of fungal pathogens were further compared with other common fatty acids, a broader screening for their potentials in antifungal activity. Appropriate amount of each individual fatty acid was dissolved in 10 ml acetone, and 1 of the 10 ml acetone–fatty acid solution was then added in 100 ml PDA medium (40–50°C) to give the final concentrations of 0, 100, 1,000, or 2,000 μmol/l, and additional concentrations of 3.9 mmol/l palmitic acid and 3.2 mmol/l oleic acid were included for the assay. Tween 20 (1‰) [13] was added in order to thoroughly mix the fatty acid with the medium while the controls only contained 1‰ Tween 20 and 1% acetone alone.

Plugs of mycelium and agar (5-mm diameter) were collected from the peripheral growth zone of the PDA sub-culture plates using a metal cork borer. Eight-day-old cultures of *A. solani, F. oxysporum* f. sp. *Cucumerinum,* and *F. oxysporum* f. sp. *lycopersici* and 14-day-old culture of *C. lagenarium* were used for getting the mycelial plug. Then these plugs were reversely placed on the center of each Petri dish (90 mm diameter) filled with 25 ml PDA medium [8] and incubated in darkness at 25°C. Each treatment had six replications.

Because of the difference in growth rate of the fungi, we measured the diameter of the fungi colony excluding the inoculum plug at different periods. The diameter of *C. lagenarium* was measured at 12 days, and the other three fungi were measured at 6 days after inoculation. The diameter was measured in two directions and the average of the two was used.

Effects of Fatty Acids on Spore Germination of Pathogens

The effect of fatty acids on spore germination of *F. oxysporum* f. sp. *cucumerinum*, *F. oxysporum* f. sp. *Lycopersici*, and *C. lagenarium* was assessed on PDA medium (pH 6.50) via fatty acids amendment at the concentrations of 0, 100, 1,000, and 2,000 μmol/L and the additional concentrations of 3.9 mmol/l palmitic acid and 3.2 mmol/l oleic acid was included. Tween 20 (1‰) [13] was added in order to thoroughly mix the fatty acid with the medium while the controls only contained 1‰ Tween 20 and 1% acetone alone. A total of 100 μl of spore suspension (5×10^5 spores/ml) was transferred to each Petri dish (90 mm diameter) containing PDA medium and spread evenly by using a sterilized bent glass rod [12], and the plates were then incubated in dark at 25°C. The spores of *F. oxysporum* f. sp. *cucumerinum*, *F. oxysporum* f. sp. *Lycopersici*, and *C. lagenarium* were counted after 13, 10, and 8 h, respectively, based on our preliminary experiments. The percentage of germinating spores was determined by observing and counting a total of 100 spores in each field of three randomly selected fields of the view on each plate by means of microscope (10 × 10) [14]. The average of three counts was used for each of three Petri dishes per treatment.

Statistical Analysis

All data in each experiment were analyzed by one-way analysis of variance (ANOVA), and for each individual experiment, means in treatment were compared to those in control using Dunnett's test [15] at $P = 0.05$ and $P = 0.01$, respectively (SPSS, 13.0).

Results

Plant Growth in Pot Experiment

The treatments of palmitic and oleic acid with the soil enhanced the growth of cucumber and tomato. The fatty acids at the high level increased root dry weights by 41% for cucumber ($F_{2,17} = 8.084$, $P = 0.004$) and 153% for tomato ($F_{2,17} = 5.087$, $P = 0.021$). Meanwhile, fatty acids amendment at the high level also increased shoot dry weights by 21% for cucumber ($F_{2,17} = 3.913$, $P = 0.043$) and 117% for tomato ($F_{2,17} = 5.087$, $P < 0.001$) (Table 1). At the low level, only the root dry weight of cucumber and shoot dry weight of tomato were increased by 47% and 75%, respectively (Table 1). Interestingly, the symptom of seedling death was observed, and the incidence of tomato seedling death was declined by

Table 1 Effects of palmitic acid and oleic acid on the dry weight of tomato and cucumber in pot experiment

Plant species	Index	CK	Low level	High level
Cucumber	Root dry weight(g/pot)	0.17 ± 0.009	0.25 ± 0.024**	0.24 ± 0.005**
	Shoot dry weight(g/pot)	1.12 ± 0.060	1.25 ± 0.066	1.35 ± 0.044*
Tomato	Root dry weight(g/pot)	0.03 ± 0.012	0.05 ± 0.002	0.076 ± 0.011**
	Shoot dry weight(g/pot)	0.12 ± 0.010	0.21 ± 0.016**	0.26 ± 0.019**
	Death of disease(plant/pot)	1.17 ± 0.167	0.17 ± 0.167**	0.17 ± 0.167**

Values are means (± SE) of 6 replicates. *, ** plant dry weight differ significantly at $P \leq 0.05$ and $P \leq 0.01$, respectively. Cucumber and tomato seedlings were harvested at 22 and 30 days after planting, respectively. Low level: 3.9 mmol palmitic acid and 3.2 mmol oleic acid per kg soil; High level: 7.8 mmol palmitic acid and 6.4 mmol oleic acid per kg soil; CK: without palmitic acid and oleic acid addition. The seedlings were thinned to three plants per pot approximately 10 days after planting

the fatty acids ($F_{2,17} = 12.0$, $P = 0.001$) (Table 1) although the cause for seedling death remains unknown.

Effects of Saturated Fatty Acids on Mycelial Growth of Four Pathogenic Fungi

Butyric acid at the concentrations of 1,000 and 2,000 μmol/l significantly reduced mycelial growth of all tested fungi (Table 2). At concentrations of 1,000 and 2,000 μmol/L, the mycelial growth was reduced by 50 and 61% for *A. solani*, 48 and 59% for *C. lagenarium*, 17 and 33% for *F. oxysporum* f. sp. *cucumerinum*, and 19 and 16% for *F. oxysporum* f. sp. *lycopersici*, respectively (Table 2). At the lower concentration 100 μmol/l, it only reduced the mycelial growth of *A. solani* (Table 2).

Caproic acid reduced the mycelial growth of *C. lagenarium* and *F. oxysporum* f. sp. *cucumerinum* by 41% and 37% respectively, at the level of 2,000 μmol/L. At the level of 1,000 μmol/L the mycelial growth of *F. oxysporum* f. sp. *cucumerinum* was reduced by 23% (Table 2).

The mycelial growth was inhibited by capric acid for *F. oxysporum* f. sp. *cucumerinum* at 2,000 μmol/l and for *C. lagenarium* at 1,000 and 2,000 μmol/l (Table 2). Capric acid (100 μmol/l) reduced the growth of *A. solani* and *C. lagenarium* by 27% and 46%, respectively.

At 2,000 μmol/l, caprylic acid inhibited mycelial growth of all tested fungi, and showed inhibitory effect on *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *Lycopersici* at 1,000 μmol/l (Table 2).

Lauric acid inhibited the tested fungi at 2,000 μmol/l except for *C. lagenarium*, *F. oxysporum* f. sp. *cucumerinum* at 1,000 μmol/l, and *F. oxysporum* f. sp. *lycopersici* at the levels of 100 and 1,000 μmol/l (Table 2).

Myristic acid had inhibitory effects on the growth of both *A. solani* and *F. oxysporum* f. sp. *lycopersici*, but did not inhibit the growth of *C. lagenarium* and *F. oxysporum* f. sp. *cucumerinum* (Table 2).

Palmitic acid, which only at the concentration of 3,900 μmol/l showed significant inhibitory effect on the growth of all tested fungi, reduced the growth of *A. solani, C. lagenarium, F. oxysporum* f. sp. *Cucumerinum*, and *F. oxysporum* f. sp. *lycopersici* by 42%, 52%, 40%, and 36%, respectively (Table 2).

Effects of Unsaturated FAs on Mycelial Growth of Four Pathogenic Fungi

Linoleic acid, at the concentration of 2,000 μmol/l, reduced the mycelial growth of *A. solani, F. oxysporum* f. sp. *Cucumerinum*, and *F. oxysporum* f. sp. *lycopersici* by 14%, 19%, and 16% respectively, while at 1,000 μmol/L it only reduced the growth of *F. oxysporum* f. sp. *lycopersici* by 19% (Table 2). In contrast, oleic acid at all concentrations up to 3,200 μmol/l had no apparent inhibitory effect on the mycelial growth of the four fungi (Table 2).

Effects of FAs on Spore Germination of Three Pathogenic Fungi

The effects of FAs on spore germination of *C. lagenarium*, *F. oxysporum* f. sp. *Cucumerinum*, and *F. oxysporum* f. sp. *lycopersici* were examined on PDA containing different fatty acids at various concentrations. As shown in Table 3, caprylic acid at the concentrations of 1,000 and 2,000 μmol/l

Table 2 Effects of fatty acids on mycelial growth of four plant pathogenic fungi

Fatty acid concentration (μmol/l)	Mycelial growth (mm)			
	A. solani	*C. lagenarium*	*F. oxysporum* f. sp. *cucumerinum*	*F. oxysporum* f. sp. *lycopersici*
Butyric acid				
0	44 ± 1.6	44 ± 2.6	54 ± 1.8	43 ± 1.5
100	27 ± 3.2**	38 ± 2.9	49 ± 2.4	43 ± 1.4
1,000	22 ± 2.4**	23 ± 2.7**	45 ± 1.1**	35 ± 1.7**
2,000	17 ± 1.5**	18 ± 2.4**	36 ± 1.7**	36 ± 1.8**
Caproic acid				
0	44 ± 1.3	44 ± 2.5	52 ± 1.7	45 ± 2.8
100	45 ± 2.8	47 ± 2.5	48 ± 1.5	46 ± 3.7
1,000	39 ± 1.6	47 ± 1.7	40 ± 2.1**	39 ± 2.6
2,000	39 ± 2.0	26 ± 3.9**	33 ± 1.5**	36 ± 0.7
Caprylic acid				
0	44 ± 1.3	44 ± 2.6	53 ± 1.3	45 ± 2.8
100	41 ± 1.5	50 ± 1.0	56 ± 1.4	43 ± 1.0
1,000	38 ± 1.3	37 ± 2.2	41 ± 1.7**	38 ± 2.0*
2,000	28 ± 2.2**	0 ± 0.0**	36 ± 3.3**	33 ± 1.4**
Capric acid				
0	44 ± 1.6	46 ± 2.3	53 ± 1.3	45 ± 1.6
100	32 ± 2.1**	25 ± 5.6**	50 ± 2.6	40 ± 2.2
1,000	27 ± 3.5**	0 ± 0.0**	45 ± 4.2	39 ± 1.5
2,000	16 ± 3.6**	0 ± 0.0**	38 ± 2.4**	38 ± 1.7
Lauric acid				
0	43 ± 2.6	47 ± 3.2	54 ± 2.8	45 ± 1.6
100	48 ± 1.2	51 ± 3.0	53 ± 2.2	34 ± 3.3**
1,000	48 ± 1.3	39 ± 3.5	44 ± 2.8*	33 ± 0.6**
2,000	36 ± 3.4	32 ± 2.7*	45 ± 2.2*	34 ± 3.1**
Myristic acid				
0	43 ± 2.6	44 ± 3.5	54 ± 2.7	45 ± 1.6
100	36 ± 1.0	41 ± 3.4	52 ± 2.8	37 ± 1.1**
1,000	35 ± 1.4	39 ± 3.2	48 ± 3.6	36 ± 1.6**
2,000	28 ± 1.0**	40 ± 5.6	45 ± 3.9	37 ± 1.4**
Palmitic acid				
0	45 ± 2.0	44 ± 2.6	55 ± 2.6	47 ± 1.6
100	42 ± 3.4	43 ± 4.1	51 ± 1.4	45 ± 3.4
1,000	40 ± 2.3	42 ± 4.9	50 ± 1.3	43 ± 1.0
2,000	39 ± 1.6	41 ± 0.8	52 ± 1.2	44 ± 1.3
3,900	26 ± 2.1**	21 ± 2.5**	33 ± 4.2**	30 ± 2.6**
Linoleic acid				
0	43 ± 2.2	44 ± 2.6	53 ± 1.3	45 ± 1.6
100	40 ± 1.4	49 ± 0.6	51 ± 0.8	45 ± 1.0
1,000	42 ± 1.4	47 ± 2.0	50 ± 0.9	36 ± 1.6**
2,000	37 ± 1.5**	43 ± 1.5	43 ± 0.7**	37 ± 0.9**

Table 2 continued

Fatty acid concentration (μmol/l)	Mycelial growth (mm)			
	A. solani	*C. lagenarium*	*F. oxysporum* f. sp. *cucumerinum*	*F. oxysporum* f. sp. *lycopersici*
Oleic acid				
0	45 ± 2.0	47 ± 3.2	55 ± 2.6	43 ± 1.5
100	43 ± 2.4	49 ± 4.2	56 ± 2.0	41 ± 1.2
1,000	41 ± 2.5	50 ± 2.3	51 ± 1.7	47 ± 1.1
2,000	49 ± 1.4	40 ± 2.8	51 ± 1.0	41 ± 1.5
3,200	41 ± 2.3	42 ± 1.0	51 ± 1.3	44 ± 1.0

Values are means (± SE) of six replicates. Means were compared between control and each fatty acid treatment in the column using Dunnett's test. *, ** mycelial growth differ significantly at $P \leq 0.05$ and $P \leq 0.01$respectively. Regardless of the type of fatty acids, values represent measurements taken at 6 days for *A. solani, F. oxysporum* f. sp. *cucumerinum, F. oxysporum* f. sp. *lycopersici* and 12 days for *C. lagenarium*

completely inhibited the spore germination of the three fungi. Caproic acid and capric acid also inhibited the spore germination of all pathogens tested at the concentrations of 1,000 and 2,000 μmol/l, and complete inhibition was achieved at the latter concentration (Table 3).

Capric acid inhibited the spore germination of the three tested fungi, except for *F. oxysporum* f. sp. *cucumerinum* at the concentration of 100 μmol/l (Table 3). The extent of the inhibitory effects was increased with the increase in concentration, and the spore germination was almost completely inhibited in the two higher concentrations (Table 3).

Butyric acid only showed inhibitory effect on the spore germination of the three tested fungi at the concentration of 2,000 μmol/l, and the extent of inhibitory effect is not large (Table 3).

When compared to the other two tested fungi, the spore germination of *C. lagenarium* was more sensitive to lauric acid treatment, and complete inhibition occurred when the concentration increased up to 1,000 μmol/l (Table 3) although only slight inhibition of spore germination for *F. oxysporum* f. sp. *lycopersici* was observed at the concentration of 2,000 μmol/l (Table 3).

The three fungi showed no sensitivity to palmitic acid treatment until the concentration was raised up to 3,900 μmol/l, at which palmitic acid completely inhibited spore germination of *C. lagenarium* and *F. oxysporum* f. sp. *lycopersici* and decreased spore germination of *F. oxysporum* f. sp. *cucumerinum* by 49% (Table 3).

In contrast, myristic acid and linoleic acid at concentrations up to 2,000 μmol/l and oleic acid up to 3,200 μmol/l had no inhibitory effect on spore germination of the three fungi (Table 3).

Discussion

Our previous study showed that some fatty acids in sesame seed cakes have contributed a large part to the growth promotion of cucumber seedling grown and the suppression effect on pathogens in both field experiments with a salt-affected soil where plant growth was poor without the soil amendment [11]. In the present study, another type of soil (classified as the cinnamon soil) in which plants grow poorly due to continuous monoculture of cucumber or tomato was employed to determine if addition of fatty acids could improve the growth of the tested plants. It was observed that the positive effect of fatty acids on the plant seedlings was similar, especially for that of tomato seedlings. The results of both studies suggested the potential function of fatty acids on amelioration of soil quality although the reasons are yet unknown.

The lower mortality of tomato seedlings in the pot experiment was observed in the treatment of fatty acids addition when compared to the non-treated control, indicating that palmitic acids and oleic acids may have antimicrobial activity, including induction of the plant resistance, against the soilborne plant pathogens, and it was consistent with our hypothesis

Table 3 Effects of fatty acids on spore germination of three plant-pathogenic fungi

Fatty acid (μmol/l)	Spore germination (%)		
	C. lagenarium	*F. oxysporum* f. sp. *cucumerinum*	*F. oxysporum* f. sp. *lycopersici*
Butyric acid			
0	92 ± 0.6	98 ± 0.7	97 ± 0.3
100	93 ± 0.9	98 ± 0.3	96 ± 0.3
1,000	87 ± 1.0	97 ± 0.9	95 ± 0.6
2,000	59 ± 3.5**	93 ± 0.6**	93 ± 1.5**
Caproic acid			
0	97 ± 0.6	96 ± 0.6	91 ± 1.7
100	98 ± 0.9	95 ± 1.3	86 ± 2.8
1,000	69 ± 3.9**	80 ± 2.5**	74 ± 4.7**
2,000	0 ± 0.0**	0 ± 0.0**	0 ± 0.0**
Caprylic acid			
0	95 ± 0.6	98 ± 0.6	91 ± 1.2
100	94 ± 2.5	97 ± 0.3	91 ± 0.3
1,000	0 ± 0.0**	0 ± 0.0**	0 ± 0.0**
2,000	0 ± 0.0**	0 ± 0.0**	0 ± 0.0**
Capric acid			
0	95 ± 0.6	97 ± 0.9	94 ± 0.3
100	28 ± 1.2**	98 ± 0.3	90 ± 0.6**
1,000	0 ± 0.0**	26 ± 1.8**	9 ± 1.0**
2,000	0 ± 0.0**	0 ± 0.0**	0 ± 0.0**
Lauric acid			
0	95 ± 1.9	97 ± 0.7	96 ± 1.5
100	91 ± 2.2	96 ± 0.9	95 ± 0.3
1,000	0 ± 0.0**	97 ± 0.7	93 ± 1.2
2,000	0 ± 0.0**	97 ± 0.9	89 ± 1.9**
Myristic acid			
0	95 ± 1.9	98 ± 0.6	96 ± 1.5
100	96 ± 0.3	97 ± 0.6	96 ± 0.9
1,000	94 ± 0.9	95 ± 1.7	97 ± 1.2
2,000	91 ± 2.7	97 ± 0.9	95 ± 1.2
Palmitic acid			
0	94 ± 0.6	98 ± 1.0	90 ± 1.5
100	94 ± 0.3	97 ± 0.9	90 ± 0.6
1,000	96 ± 1.2	97 ± 0.6	89 ± 1.2
2,000	94 ± 1.2	97 ± 0.9	88 ± 1.0
3,900	0 ± 0.0**	50 ± 3.6**	0 ± 0.0**
Linoleic acid			
0	96 ± 1.8	97 ± 1.0	98 ± 0.3
100	95 ± 1.2	97 ± 0.6	98 ± 0.6
1,000	98 ± 0.7	99 ± 0.3	97 ± 0.6
2,000	94 ± 1.0	98 ± 1.3	98 ± 0.6

Table 3 continued

Fatty acid (μmol/l)	Spore germination (%)		
	C. lagenarium	*F. oxysporum* f. sp. *cucumerinum*	*F. oxysporum* f. sp. *lycopersici*
Oleic acid			
0	94 ± 0.6	97 ± 0.7	93 ± 1.0
100	94 ± 2.5	97 ± 1.2	92 ± 1.0
1,000	96 ± 0.3	98 ± 0.3	91 ± 0.6
2,000	97 ± 0.6	97 ± 1.2	92 ± 1.5
3,200	92 ± 1.5	98 ± 0.3	95 ± 1.2

Values are means (±SE) of three replicates. Means were compared between control and each fatty acid treatment in the column using Dunnett's test. *, ** the percentage of germinated spores differ significantly at $P \leq 0.05$ and $P \leq 0.01$, respectively, from the control. Regardless of the type of fatty acids, the germinated spores were counted at 10 h for *F. oxysporum* f. sp. *Lycopersici*, 13 h for *F. oxysporum* f. sp. *cucumerinum* and 8 h for *C. lagenarium*

that fatty acids in the sesame cake crude extracts are responsible for plant growth enhancement, through suppression of plant pathogens. Consequently, we then analyzed the effect of nine fatty acids on the mycelial growth and spore germination of four fungi that are known soil-borne pathogens causing severe diseases of a number of crops [16]. Surprisingly, it was observed that butyric acid, caproic acid, and caprylic acid showed promising antifungal activity against the phytopathogens in the present experiment, and a strong inhibitory effect of caprylic acid against the spore germination was also observed.

With the exception of oleic acid, the tested fatty acids really had the characteristic of antifungal activity via negative impacts either on the spore germination or mycelial growth or both which were dependent on the types of fungi and acids. Even at the lower level of 100 μmol/l, capric acid already inhibited two of the tested fungi, suggesting that this fatty acid is of great potential to control plant-pathogenic fungi. Caproic acid, caprylic acid, capric acid, and palmitic acid almost completely inhibited the spore germination of all three tested fungi at higher concentrations, indicating their relative broad antifungal activities.

The findings that saturated fatty acid—palmitic acids—had a higher antifungal activity than the unsaturated fatty acid oleic acid are contrary to the findings of Walters et al. [8] that the monoene- and polyene-unsaturated fatty acid showed antifungal activity at 100 or 1,000 μmol/l or both [8]. The antifungal activity of palmitic acids might have a partial contribution to the lower mortality of tomato seedling in the pot experiment.

The results showed that capric acid could completely inhibit the mycelial growth of *C. lagenarium* and spore germination of *C. lagenarium*, *F. oxysporum* f. sp. *Cucumerinum*, and *F. oxysporum* f. sp. *lycopersici* at higher concentrations; this is consistent with the previous findings that capric acid caused the fastest and most effective killing of three strains of *Candida albicans* [4]. However, the sensitivities of the fungi tested in this study to capric acid at lower concentration appear to be much higher when compared to *Candida albicans* [4]. Lauric acid also showed the inhibitory effect against the spore germination of *C. lagenarium* at 1,000 μmol/l in this study, but the lowest concentration of lauric acid causing complete lysis of the cells of *Candida albicans* was 2,500 μmol/l in a previous study [4].

While linoleic acid inhibited the mycelial growth of *A. solani*, *F. oxysporum* f. sp. *Lycopersici*, and *F. oxysporum* f. sp. *cucumerinum* at 2,000 μmol/l, but it already exhibited an antifungal activity against *Crinipellis perniciosa* at the lower level of 100 μmol/l [8]. Neither the spore germination nor mycelial growth of all tested fungi was affected by oleic acid; this is contrary to the work that oleic acid caused significant reductions in mycelial growth of *Pythium ultimum* and *Crinipellis perniciosa* at 1,000 μmol/l [8]. We speculated that the difference in fungal species might be responsible for this discrepancy, and further study should be performed including the fungi in both experiments. In addition, other possible mechanisms,

such as direct plant growth promotion that resulted in a more healthy and resistant plant and change of soil microbial communities might be responsible; it deserves further investigation.

The antifungal activities of many fatty acids and possible mechanisms of antifungal activity have been previously studied. One of the mechanisms focused on fungal membrane disruption [4, 5]. For example, it has been demonstrated that capric acid caused the greatest reduction of *Candida albicans* due to a disrupted or disintegrated plasma membrane caused by a hydrostatic turgor pressure within the cell [4], and the similar mechanism has also been found in the antifungal activity of (Z)-9-heptadecenoic acid against the fungi *Phytophthora infestans* and *Idriella bolleyi* [5]. Moreover, interfering fungal sphingolipid biosynthesis has also been suggested to be an antifungal activity of acetylenic fatty acid [17]. Up to date, most studies of the antifungal mechanisms of fatty acids were focused on the inhibitory effect against human pathogenic fungi, whereas the antifungal activities against phytopathogens are still unknown.

In summary, the present study provides some information of fatty acids against four economically important plant-pathogenic fungi. It is necessary to conduct further research on the mechanism by which the fatty acids take action on controlling the pathogenic fungi. The potential for fatty acids to inhibit plant pathogens in situ needs further study. Because fatty acids are the kind of natural and friendly environmental products, they might offer new strategies to control plant-pathogenic fungi in future sustainable agriculture.

Acknowledgments We are grateful to Dr. Yaoting Yu (Nankai University, China) and Dr. Senyu Chen (University of Minnesota, USA) for their critical review of this manuscript. We also thank Dr. Zhijin Fan (Nankai University, China) and Dr. Ligang Zhou (China Agricultural University, China) for providing tested fungi and helpful suggestions. This work was jointly supported by Tianjin Natural Science foundation (06YFJMJC121000), National High-tech R&D Program (2006AA10Z423) and National Key Technologies R&D Program (2006BAD07B03).

References

1. Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP. Fatty acids and derivatives as antimicrobial agents. Antimicrob Agent Chemother. 1972;2:23–8.
2. Carballeira NM. New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents. Prog Lipid Res. 2008;47:50–61.
3. Morbidoni HR, Vilcheze C, Kremer L, Bittman R, Sacchettini JC Jr, Jacobs WR. Dual inhibition of mycobacterial fatty acid biosynthesis and degradation by 2-alkynoic acids. Chem Biol. 2006;13:297–307.
4. Bergsson G, Arnfinnsson J, Steingrimsson O, Thormar H. In vitro killing of *Candida albicans* by fatty acids and monoglycerides. Antimicrob Agents Chemother. 2001;45:3209–12.
5. Avis TJ, Bélanger RR. Specificity and mode of action of the antifungal fatty acid cis-9-heptadecenoic acid produced by *Pseudozyma flocculosa*. Appl Environ Microbiol. 2001;67:956–60.
6. Walters DR, Walker RL, Walker KC. Lauric acid exhibits antifungal activity against plant pathogenic fungi. J Phytopathol. 2003;151:228–30.
7. Řiháková Z, Plocková M, Filip V, Šmidrkal J. Antifungalactivity of lauric acid derivatives against *Aspergillus niger*. Eur Food Res Tech. 2001;213:488–90.
8. Walters D, Raynor L, Mitchell A, Walker R, Walker K. Antifungal activities of four fatty acids against plant pathogenic fungi. Mycopathologia. 2004;157:87–90.
9. Duniway JM. Status of chemical alternatives to methyl bromide for pre-plant fumigation of soil. Phytopathology. 2002;92:1337–43.
10. Ruan WB, Liu MH, Pan J, Wang J, Lu WL, Ma CC, Wang JG, Gao YB. Effect of oil cakes on the growth of cucumber (*Cucumis sativus* L.) under continuous cropping system and its mechanism. Scientia Agricultura Sinica. 2003;36(12):1519–24.
11. Ruan WB, Wang J, Pan J, Li HB, Wang JG, Zhang FS, Gao YB. Effects of sesame seed cake allelochemicals on the growth cucumber (*Cucumis sativus* L. cv. Jinchun 4). Allelopathy J. 2005;16(2):217–25.
12. Liu XP, Wang JY, Gou P, Mao CG, Zhu ZR, Li HY. In vitro inhibition of postharvest pathogens of fruit and control of gray mold of strawberry and green mold of citrus by aureobasidin A. Int J Food Microbiol. 2007;119:223–9.
13. Ross WF, Walters DR, Robins DJ. Synthesis and antifungal activity of five classes of diamines. Pest Manag Sci. 2004;60:143–8.
14. Widmer TL, Laurent N. Plant extracts containing caffeic acid and rosmarinic acid inhibit zoospore germination of Phytophthora spp. pathogenic to *Theobroma cacao*. Eur J Plant Pathol. 2006;115:377–88.
15. Karuna R, Sashidhar RB. The mycotoxin fumonisin B1 inhibits eukaryotic protein synthesis: in vitro and in vivo studies. Mycopathologia. 2008;165:37–49.
16. Agrios GN. Plant pathology. 4th ed. San Francisco: Academic Press; 1997.
17. Li XC, Jacob MR, ElSohly HN, Nagle DG, Smillie TJ, Walker LA, et al. Acetylenic acids inhibiting azole-resistant *Candida albicans* from *Pentagonia gigantifolia*. J Nat Prod. 2003;66:1132–5.