JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Potential Antagonistic Effects of Nine Natural Fatty Acids Against *Meloidogyne incognita*

Wei-pu Zhang, Wei-bin Ruan,* Yun-ying Deng, and Yu-bao Gao

College of Life Sciences, Nankai University, Tianjin 300071, P. R. China

ABSTRACT: Fatty acids, the essential components of life, were widely present in various seed cakes, gutter oil, and other resources. The objective of this study was to evaluate the potential antagonistic effects of nine fatty acids (FAs) against *Meloidogyne incognita* (root-knot nematodes). The results showed that butyric, caprylic, capric, lauric, myristic, palmitic, and oleic acids significantly reduced *M. incognita* reproduction, whereas cucumber (*Cucumus sativus*) biomass was not adversely affected by the tested FAs and was even significantly increased in several fatty acids treatments. All nine tested fatty acids showed apparent inhibitory effects on egg hatching on day 21, especially capric acid with which the hatching rate was reduced to 15.8% as compared to that using sterile distilled water. Caproic, caprylic, capric, lauric, myristic, and palmitic acids caused significantly higher mortality of the second-stage juvenile of *M. incognita* than the other three FAs, and both caprylic and capric acids resulted in approximately 50% mortality (2000 μmol/L) after a 24 h exposure. In conclusion, fatty acids showed the nematicidal effect differently, among which capric acid showed a strong nematicidal effect and might be a powerful active substance for integrated *M. incognita* management. Given the general nematicidal properties of FAs, farmers might utilize waste resources, such as oil seed cake, gutter oil, etc., containing various FAs or use pure FAs for effective *M. incognita* management.

KEYWORDS: *mortality, plant parasitic nematodes, seed cakes, biocontrol, nematicide*

INTRODUCTION

Fatty acids (FAs) are ubiquitous in nature and play crucial roles in life processes because they are basic components of lipids and occur naturally in the form of glycerol esters in animal or vegetable fats and oils. Most naturally occurring FAs contain a chain with an even number of carbon atoms, ranging from 4 to 28. A large number of FAs have antifungal activities,[1−3] including butyric, caproic, caprylic, capric, and palmitic acids, while some unsaturated FAs have an inhibitory effect on several plant pathogens. Unsaturated FAs activate resistance against plant diseases such as protecting pearl millet from downy mildew.[4] In addition, several types of FAs and their derivatives have also been reported to have nematicidal activities.[5−7] Djian et al.[5] reported that volatile FAs (i.e., acetic acid, propionic acid, and butyric acid) had nematotoxic activity against *Meloidogyne arenaria*. Unsaturated FAs had the same or greater activity than saturated FAs with the same carbon numbers. Schwarz et al.[6] showed that 3-hydroxypropionic acid was active against *Meloidogyne incognita* and *Caenorhabditis elegans*. Davis et al.[7] reported that the microemulsions of the C_9 FA esters inhibited *Meloidogyne javanica* and *Heterodera glycines*. Several other studies have also demonstrated the nematicidal activities of some fatty acids from different natural sources. A methanol extract from the ripe fruits of *Melia azedarach*, containing caproic acid (C6:0 FAs) and others, had high nematicidal activity against the second stage juveniles of *M. incognita*.[8,9] Volatile FAs released from the anaerobic digestion of animal manures also showed the nematicidal activities against *H. glycines*.[10,11] Furthermore, both pentadecanoic and palmitic acids from the bacterium *Lysinibacillus mangiferahumi* were shown to have nematicidal activities against *M. incogntia*.[12]

Fatty acids are present in wastes or byproducts of food industry. Traditionally, a large amount of seed cakes are applied to agricultural fields as an organic amendment worldwide. In seed cakes various fatty acids have been detected,[13−15] which include palmitic, oleic, stearic, and linoleic acids present in oil from soybeans, sunflower seed, maize, rape,[16] and pomegranates.[17] In addition to improvement of soil fertility, seed cake has a potential effect on the control of plant parasitic nematodes and other plant pathogens.[18] However, in most cases application of seed cakes is for the purpose to improve soil fertility, rather than for the control of plant parasitic nematodes and plant pathogens, which is the intrinsic properties of seed cakes. In addition, it is estimated that 2−3 million tons of kitchen waste, namely cooking oil waste, is recycled annually in China and even misused in restaurants, hotels, and public canteens. Gutter oil contains many carcinogenic substances.[19] Therefore, exploration of other optional ways for the reuse of gutter oil is urgent and necessary with regard to human health.

Root-knot nematodes (RKNs) (*Meloidogyne* spp.) are one of the most damaging nematodes causing an estimated annual loss of $118 billion U.S. to world crop yields.[20] In particular, the *Meloidogyne* spp. is an obligate parasite with a broad host spectrum, which infects approximately 1700 plant species.[21] Currently, the main option for the control of plant parasitic nematodes is the application of chemical nematicides and fumigants, which may have negative environmental side effects. Thus, there is an increasing need to develop environmentally safe and effective alternative strategies for the control of plant parasitic nematodes, which could replace nematicides and fumigants that have been abandoned or restricted due to

Received: August 29, 2012
Revised: November 2, 2012
Accepted: November 3, 2012
Published: November 3, 2012

ACS Publications © 2012 American Chemical Society

dx.doi.org/10.1021/jf3036885 | J. Agric. Food Chem. 2012, 60, 11631−11637

potential hazard to the environment or human health.[22] For instance, neem oil cake was shown to be effective in the management of plant parasitic nematodes, such as *Pratylenchus delattrei* with increased plant biomass and higher flower yields.[23] There have been 36 natural organic compounds identified and evaluated for their potential use in nematode control.[24]

Considering the nematicidal properties of fatty acids, we hypothesized that these byproducts and wastes (seed cake, gutter oil, and other sources) might have the potential for RKNs control. Previous studies have been conducted on the antagonistic effects of seed cake against the *Meloidogyne* spp.,[18,25,26] but direct evaluations of common FAs against *M. incognita* are quite few. In addition, previous assessments of the nematicidal activity of FAs have only tested one or two FAs simultaneously, despite that fact, simultaneous evaluation of the nematicidal properties of several fatty acids can give insight on the future use of these byproducts and wastes for plant parasitic nematode control.

The objective of the present study was to simultaneously evaluate the effects of seven saturated FAs, namely, butyric, caproic, caprylic, capric, lauric, myristic, and palmitic acids, as well as two unsaturated FAs, oleic and linoleic acids, on reproduction, egg hatching, and J2 mortality of *M. incognita*.

MATERIALS AND METHODS

Fatty Acids. The saturated FAs tested for their nematicidal activities included butyric (C4:0 95% purity), caproic (C6:0 99% purity), caprylic (C8:0 98% purity), capric (C10:0 99% purity), lauric (C12:0 98% purity), myristic (C14:0 99% purity), and palmitic (C16:0 99% purity) acids, and the unsaturated fatty acids were oleic acid ($C18{:}1\Delta^{9c}$ 99% purity) and linoleic acid ($C18{:}2\Delta^{9c,12c}$ 97% purity). All reagents were purchased from the Tianjin Reagent Corp.

An appropriate amount of each FA was dissolved and thoroughly mixed with 10 mL acetone, 1 mL Tween-80, and 1 mL dimethyl sulfoxide, which was subsequently added to an acetone/FA mixture and mixed evenly. The Tween-80/dimethyl sulfoxide/acetone mixture was regarded as a medium control (Medium) in the following assays. Next, 1 mL of the Tween-80/dimethyl sulfoxide/acetone/FA solution was diluted with sterile distilled water (SDW) to make a solution of 100 mL (2000 μmol/L). An appropriate volume of solution was diluted with SDW to give final concentrations of 100 and 1000 μmol/L.

Nematode Preparation. Eggs were obtained from the *M. incognita*-infected pepper (*Capsicum annuum* L.) plants cultured under greenhouse conditions by stirring with a 1% NaClO solution, rinsing thoroughly with tap water, and centrifuging with a 38% sucrose solution. The supernatant was rinsed with tap water and SDW, and the prepared egg solutions were used within one day. [27]

The *M. incognita* J2s were hatched from the egg masses and were placed in an antibiotic SCQ solution (100 ppm streptomycin sulfate, 50 ppm chlortetracycline, and 20 ppm 8-quinolinol) for 24 h at 4 °C[28] and then rinsed with SDW.

Effects of Fatty Acids on *M. incognita* Reproduction. A pot assay was conducted in a greenhouse at the College of Life Sciences, Nankai University. Cucumber seeds (*Cucumis sativus* var. Jinchun No. 4) were purchased from the Tianjin Academy of Agricultural Sciences. After heating at 80 °C for 8 h, 0.8 kg of a soil and vermiculite mixture (1:1 v/v) was placed into each pot and fertilized with 0.74 g K_2SO_4, 0.74 g $CO(NH_2)_2$, and 0.48 g $NH_4H_2PO_4$ per kg of soil mixture.[2] Next, eight FAs were added separately (i.e., butyric acid, caproic acid, caprylic acid, lauric acid, myristic acid, palmitic acid, and oleic acid) and thoroughly mixed with the soil mixture at 0.15‰ w/w or 1.5‰ w/w. Pots without FAs were included as controls. A single one-week-old cucumber plant was transplanted into each pot. Four days after transplantation, 2 mL egg suspensions (5000 eggs) were inoculated into four holes (1−2 cm depth) around the base of each cucumber plant. Pots were arranged randomly and watered as necessary. There were five pots for each FA treatment and 10 pots for the controls (50 pots in total). The cucumber plants were harvested 40 days after *M. incognita* inoculation. The *M. incognita* eggs were collected from the plant roots as previously described and counted using an inverted microscope (CK41 Olympus). Both shoots and roots where eggs were collected were oven-dried at 105 °C for 30 min, followed by 80 °C for 10 h, and finally, they were weighed. The plant height, leaf number, stem diameter, and shoot and root dry weights were recorded. The experiments were repeated once.

Hatching Assay. The effects of FAs on the hatching of *M. incognita* were quantified based on the published procedures described in literature.[28] We pipetted 1 mL of a suspension containing 500 eggs onto a 1 cm diameter sieve with a 35 μm pore size. The sieves containing eggs were placed individually into the single well of a 24-well tissue culture plate, where only the center well of each plate was used.[11] Approximately 2 mL prepared FAs (100, 1000, and 2000 μmol/L) were added to the tissue culture plates so it only reached the bottoms of the sieves. SDW and Medium were included as controls. Each tissue culture plate included one treatment with six replicates to avoid disturbance among treatments, and plates were sealed with parafilm. The tissue culture plates were incubated in an incubator at 25 °C. On day 3, 7, 14, and 21 after exposure, the hatching solutions were replaced with the fresh solution, and the J2s that hatched in the solutions were counted.

J2 Viability. This experiment was performed with 24-well tissue culture plates. We poured 1 mL of prepared FAs (0, 100, 1000, and 2000 μmol/L) into tissue culture plates, and then added 20 μL of the nematode suspension (i.e., 50 to 100 nematodes). Each tissue culture plate included one treatment with six replicates. Plates were covered with plastic lids and sealed with parafilm to minimize evaporation and the loss of solution, followed by dark incubation at 25 °C. The mortality response was determined after 12 and 24 h of exposure at three different concentrations. After the incubation period, the mortality of J2s was quantified using an inverted microscope (CK41 Olympus). The viability of J2s was determined by adding 80 μL of NaOH to the solution, and J2s that changed shape from straight to curled or hook-shaped within 1 min were not considered viable.[29] Each treatment was repeated once with six replications.

Statistical Analysis. All data from the assays of *M. incognita* reproduction, egg hatching, and J2 viability were analyzed using a one-way analysis of variance (ANOVA). The egg number and plant biomass in the reproduction assay were log-transformed to satisfy the ANOVA assumptions of homogeneity of variances. The percentage data for J2 viability were arcsine-transformed to meet the ANOVA assumptions of homogeneity of variances.

The means of data from the reproduction and egg hatching assay were compared using the Fisher's least significant difference test at $P < 0.05$. In the J2 viability test, each FA was compared to the control (medium solution) using the Dunnett's test at $P < 0.05$ and $P < 0.01$. All data were analyzed using SPSS 16.0.

RESULTS

Effects on *M. incognita* Reproduction and Plant Biomass. The effect of FAs application on egg number was significantly different in trial 1 ($F_{16,73} = 5.076$, $P < 0.001$) and trial 2 ($F_{16,73} = 2.688$, $P = 0.002$) (Figure1). In this trial, butyric (0.15‰), caprylic (0.15‰), capric (0.15‰, 1.5‰), Lauric (1.5‰), myristic (0.15‰, 1.5‰), palmitic (1.5‰), and oleic acids (0.15‰, 1.5‰) caused a significant reduction of nematode reproduction as compared with the control. In the second trial, caproic (1.5‰) caprylic (1.5‰), capric (0.15‰, 1.5‰), and oleic acids (1.5‰) showed significant adverse effects on nematode production. In both trials, plant biomass was not affected by the addition of FAs (Figure 2). The plant growth enhancement in Trial 1 ($F_{16,73} = 3.276$, $P < 0.001$) seemed to be higher than that in trial 2 ($F_{16,73} = 1.384$, $P = 0.174$). In the former, lauric (0.15‰, 1.5‰), palmitic

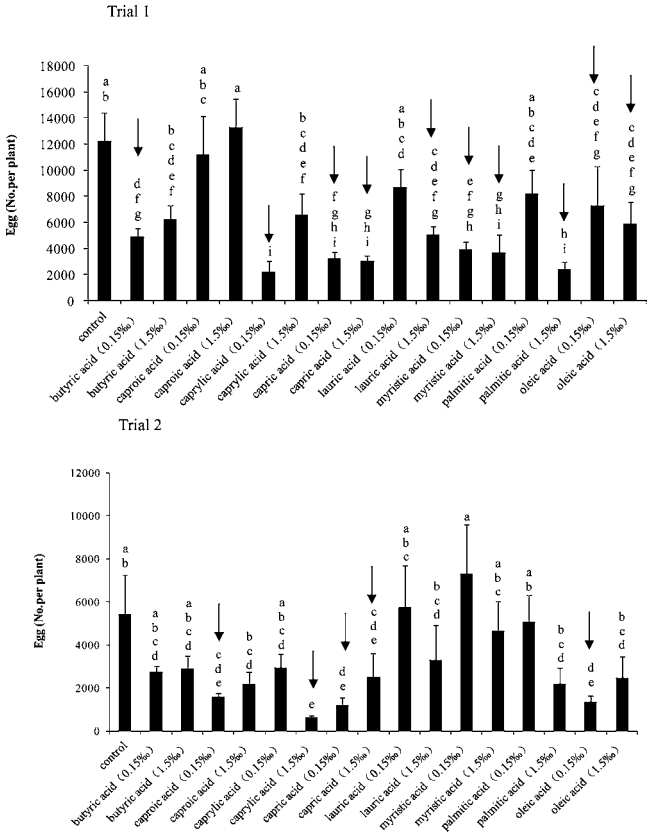

Figure 1. The effects of eight fatty acids on egg numbers of *M. incognita* in cucumber (Trials 1 and 2). Fatty acids were premixed with soil at the rate of 0.15‰ and 1.5‰ (w/w). There were five pots for each fatty acid treatment and 10 pots for controls. The whole experiment was repeated once. Different letters indicate a significant difference between treatments ($P < 0.05$). The arrow above bar indicates the significant difference between the control and fatty acid treatment ($P < 0.05$).

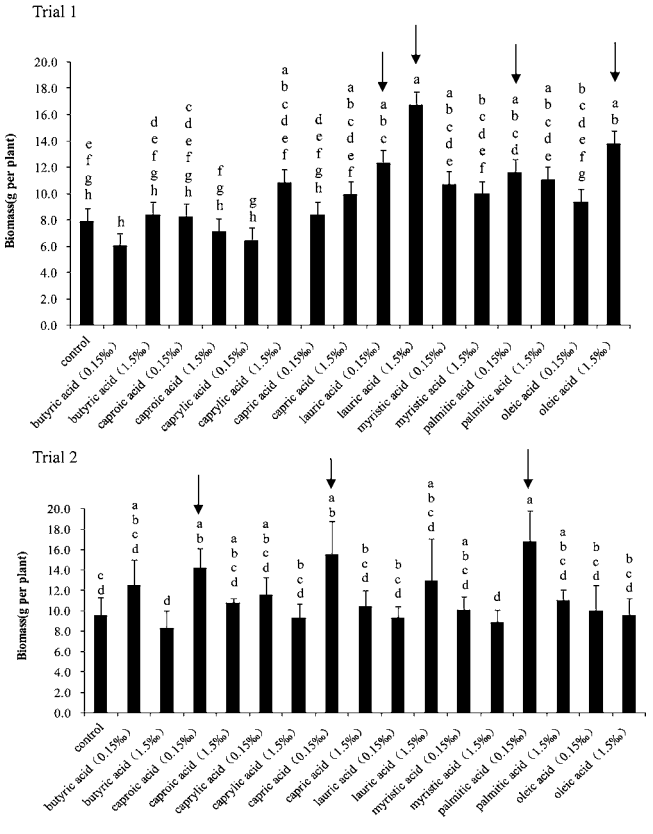

Figure 2. The effects of eight fatty acids on the biomass of *M. incognita* in cucumber (Trials 1 and 2). Fatty acids were premixed with soil at the rate of 0.15‰ and 1.5‰ (w/w). There were five pots for each fatty acid treatment and 10 pots for controls. The whole experiment was repeated once. Different letters indicate a significant difference between treatments ($P < 0.05$). The arrows above bars indicate the significant difference between the control and fatty acid treatment ($P < 0.05$).

(0.15‰), and oleic (1.5‰) acids even showed a significant stimulation to plant growth while in the latter, plant growth was improved with capric (0.15‰) and palmitic (0.15‰) acids compared to the control.

Effects on Hatching. In general, the inhibitory effects of fatty acids on egg hatching were observed on day 14 and 21 after exposure. In particular, egg hatch rate in capric acid (2000 μmol/L) was significantly reduced to 13.4% on day 14 and 15.8% on day 21 as compared to that of the SDW control and to 15.8% on day 14 and 18.0% on day 14 as compared to that of the Medium control (Table 1).

Effects on J2 Mortality. In general, fatty acids exposure had apparent effects on the J2 mortality (Table 2). Compared to the control, the J2 mortality of *M. incognita* after 12 and 24 h exposure to caprylic, capric, and lauric acids was significantly higher at 1000 μmol/L and 2000 μmol/L in both trials, respectively. In addition, the J2 morality in caproic (2000 μmol/L) and palmitic acid (2000 μmol/L) was also significantly higher than those of their corresponding control treatment.

DISCUSSION

The present study clearly demonstrated the presence of nematicidal effects of tested fatty acids, in terms of reproduction, egg hatching, and J2 mortality of *M. incogntia* RKNs, although the suppression varied with types of FAs. It indicated that natural fatty acids, capric acid in particular, might have the potential to contribute to the integrated RKNs management.

In general, most of the tested fatty acids showed the trend of inhibiting the reproduction of RKNs. Capric (C10:0) (0.15‰, 1.5‰), and oleic acid (C18:1Δ^{9c}) (0.15‰) significantly inhibited RKN production in both trials and did not show phytotoxic effects. Rather, a stimulatory effect was observed in the treatment with palmitic acids (0.15‰). From the economic perspective, the low level of capric acid (C10:0) (0.15‰) with clear antagonistic effects against RKNs will decrease the treatment costs, which could make a more competitive candidate for further exploration as an alternative to chemical nematicides or fumigants. For example, the positive results of reproduction depression, egg hatching inhibition, and J2 mortality showed that capric acid could be a potential alternative for *M. incognita* control. To our knowledge, this is the first report of possible nematicidal properties of capric acid for RKN control. Past research reported that butyric acid was effective as a synthetic nematicide in suppressing *M. incognita*,[30] and *Pratylenchus penetrans*.[31] β-amino-butyric acid, a derivative of butyric acid (C4:0), also had an inhibitory effect on root-know nematodes (*M. javanica*).[32] In the present study, butyric acid manifested the inhibitory effects on RKNs, but only reached the significant level at the level of 0.15‰ in the first

Table 1. Cumulative Percentage of Hatching of *Meloidogyne incognita* Treated with Nine Fatty Acids[a]

fatty acids (μmol/L)		3 d	7 d	14 d	21 d
butyric acid	100	1.60 ± 1.28	6.70 ± 1.89ab	**13.90 ± 3.69b**	**20.37 ± 3.93b**
	1000	2.00 ± 1.41	7.87 ± 2.64ab	**17.17 ± 4.22b**	**21.47 ± 4.25b**
	2000	0.67 ± 0.43	4.47 ± 2.05b	**8.17 ± 3.55b**	**14.13 ± 3.86b**
SDW		0.57 ± 0.37	12.63 ± 3.84a	35.77 ± 4.48a	44.87 ± 3.93a
medium		0.20 ± 0.09	10.40 ± 1.37ab	30.47 ± 2.12a	39.23 ± 1.44a
caproic acid	100	0.87 ± 0.21b	2.97 ± 0.37bc	**9.57 ± 1.77b**	**15.97 ± 2.70b**
	1000	2.77 ± 0.69a	7.67 ± 2.03ab	**16.67 ± 3.52b**	**21.20 ± 3.61b**
	2000	0.80 ± 0.12b	6.40 ± 1.35b	**14.40 ± 2.43b**	**19.67 ± 2.53b**
SDW		0.57 ± 0.37b	12.63 ± 3.84a	35.77 ± 4.48a	44.87 ± 3.93a
medium		0.20 ± 0.09b	10.40 ± 1.37ab	30.47 ± 2.12a	39.23 ± 1.44a
caprylic acid	100	2.30 ± 1.38ab	8.60 ± 3.80a	23.63 ± 4.27bc	30.93 ± 3.13bc
	1000	2.60 ± 0.92a	9.03 ± 2.75a	**16.87 ± 3.66c**	**21.00 ± 4.45 cd**
	2000	0.73 ± 0.31ab	5.97 ± 1.51a	**13.07 ± 3.72c**	**15.07 ± 4.11d**
SDW		0.57 ± 0.37ab	12.63 ± 3.84a	35.77 ± 4.48a	44.87 ± 3.93a
medium		0.20 ± 0.09b	10.40 ± 1.37a	30.47 ± 2.12ab	39.23 ± 1.44ab
capric acid	100	0.43 ± 0.14	4.73 ± 0.88bc	**19.57 ± 0.45bc**	**25.93 ± 0.75b**
	1000	0.97 ± 0.73	4.77 ± 3.37bc	**11.50 ± 3.98 cd**	**16.40 ± 3.73c**
	2000	0.23 ± 0.06	2.07 ± 1.16c	**4.80 ± 2.69d**	**7.10 ± 3.08d**
SDW		0.57 ± 0.37a	12.63 ± 3.84a	35.77 ± 4.48a	44.87 ± 3.93a
medium		0.20 ± 0.09	10.40 ± 1.37ab	30.47 ± 2.12a	39.23 ± 1.44a
lauric acid	100	0.50 ± 0.09b	4.73 ± 1.16b	**20.10 ± 1.44b**	**27.10 ± 1.32b**
	1000	3.67 ± 1.39a	12.70 ± 2.70a	**20.37 ± 2.77b**	**24.17 ± 2.73b**
	2000	1.60 ± 0.82ab	7.57 ± 1.65ab	**17.47 ± 1.35b**	**20.77 ± 1.97b**
SDW		0.57 ± 0.37b	12.63 ± 3.84a	35.77 ± 4.48a	44.87 ± 3.93a
medium		0.20 ± 0.09b	10.40 ± 1.37ab	30.47 ± 2.12a	39.23 ± 1.44a
myristic acid	100	1.80 ± 1.56	8.87 ± 3.74a	**18.40 ± 2.95b**	**27.67 ± 2.73b**
	1000	0.37 ± 0.10	1.47 ± 0.23b	**12.93 ± 1.88b**	**23.97 ± 4.24b**
	2000	0.27 ± 0.07	1.53 ± 0.47b	**12.00 ± 1.75b**	**20.00 ± 2.82b**
SDW		0.57 ± 0.37	12.63 ± 3.84a	35.77 ± 4.48a	44.87 ± 3.93a
medium		0.20 ± 0.09	10.40 ± 1.37a	30.47 ± 2.12a	39.23 ± 1.44a
palmitic acid	100	1.13 ± 0.70	5.73 ± 3.36ab	**15.07 ± 3.51b**	**22.30 ± 2.63b**
	1000	0.23 ± 0.08	2.80 ± 0.56b	**9.53 ± 1.30b**	**13.40 ± 1.20c**
	2000	0.70 ± 0.37	6.63 ± 1.56ab	**17.23 ± 2.38b**	**20.17 ± 1.91bc**
SDW		0.57 ± 0.37	12.63 ± 3.84	35.77 ± 4.48a	44.87 ± 3.93a
medium		0.20 ± 0.09	10.40 ± 1.37	30.47 ± 2.12a	39.23 ± 1.44a
oleic acid	100	1.47 ± 1.08	6.07 ± 3.46	**16.60 ± 3.60b**	**24.93 ± 2.16b**
	1000	0.67 ± 0.21	6.03 ± 1.00	**18.53 ± 2.10b**	**25.57 ± 1.59b**
	2000	1.23 ± 0.74	7.47 ± 1.99	**16.53 ± 1.53b**	**21.80 ± 1.48b**
SDW		0.57 ± 0.37	12.63 ± 3.84a	35.77 ± 4.48a	44.87 ± 3.93a
medium		0.20 ± 0.09	10.40 ± 1.37a	30.47 ± 2.12a	39.23 ± 1.44a
linoleic acid	100	0.33 ± 0.11	6.17 ± 1.31bc	29.80 ± 2.80ab	37.97 ± 3.08ab
	1000	0.13 ± 0.04	6.60 ± 2.16abc	20.43 ± 5.66b	29.47 ± 5.27b
	2000	0.33 ± 0.14	2.30 ± 0.59c	**5.87 ± 1.47c**	**11.33 ± 2.13c**
SDW		0.57 ± 0.37	12.63 ± 3.84a	35.77 ± 4.48a	44.87 ± 3.93a
medium		0.20 ± 0.09	10.40 ± 1.37a	30.47 ± 2.12a	39.23 ± 1.44ab

[a]The concentrations were 0, 100, 1000, and 2000 μmol/L on Day 3, 7, 14, and 21, respectively. Sterile distilled water (SDW) and the medium for dissolving fatty acids were included as controls, respectively. Data are means ± SE with 6 replicates. For each of nine fatty acids, sterile distilled water control and medium control were included. The values followed by a different letter in a column for each fatty acid were significantly different at $P < 0.05$.

trial. The difference in medium, the sand in Browning's study, and the mixture of soil and vermiculate in this study might partially contribute to the discrepancy between the two studies.[30] Considering that the nematicidal effects of butyric acid on RKNs appear to be lower than those of capric acid in the present study, we predicted that the latter might have higher potential for RKN control.

It was interesting to note that palmitic acid (1.5‰ in the first trial) and oleic acid (0.15‰ in both trials, 1.5‰ in the first trial) showed their nematicidal activity. Palmitic acid, oleic acid, and linoleic acid extracted from the stem and root of *Mucuna aterrima* have demonstrated nematicidal activity due to their ability of killing *M. incognita* J2s and inhibiting egg hatching.[33] Seo et al.[34] suggested that the existence of a double bond at the α,β-position of the carbonyl group might increase the nematicidal activity of aldehydes. This may be said of oleic acid (C18:1Δ^{9c}) because it possesses a double bond. Our previous study found that palmitic (C16:0) and oleic acid (C18:1Δ^{9c}) were the main components of sesame cake.[35] Given that these two acids are the basic components of seed

Table 2. Percent Mortality of the Second-Stage Juveniles of *Meloidogyne incognita* Treated with Nine Fatty Acids[a]

fatty acid concentration		trial 1		trial 2	
	(μmol/L)	12 h	24 h	12 h	24 h
butyric acid	0	1.79 ± 0.52	2.51 ± 0.66	3.30 ± 0.67	1.47 ± 0.54
	100	4.02 ± 1.24	4.76 ± 0.72	2.55 ± 0.65	2.34 ± 0.39
	1000	**6.60 ± 1.64***	**7.55 ± 0.68****	2.91 ± 0.50	3.23 ± 0.75
	2000	**8.63 ± 1.66****	**6.99 ± 1.85***	3.34 ± 0.60	3.28 ± 0.73
caproic acid	0	0.95 ± 0.42	2.22 ± 1.11	3.30 ± 0.67	1.47 ± 0.54
	100	0.39 ± 0.25	5.35 ± 1.37	2.54 ± 0.78	2.29 ± 0.41
	1000	1.50 ± 0.37	9.70 ± 4.31	3.62 ± 0.62	2.02 ± 0.46
	2000	1.09 ± 0.62	**13.32 ± 4.53***	3.78 ± 0.32	**4.44 ± 0.83***
caprylic acid	0	0.95 ± 0.42	2.22 ± 1.11	3.30 ± 0.67	1.47 ± 0.54
	100	1.65 ± 0.45	1.08 ± 0.57	3.04 ± 0.64	1.80 ± 0.46
	1000	**40.51 ± 5.32****	**65.27 ± 3.47****	**65.07 ± 2.60****	**49.88 ± 4.61****
	2000	**93.45 ± 1.17****	**66.51 ± 3.32****	**65.61 ± 6.98****	**56.35 ± 4.89****
capric acid	0	1.79 ± 0.52	2.51 ± 0.66	0.88 ± 0.50	1.96 ± 0.54
	100	**2.81 ± 0.86***	**4.42 ± 1.06**	2.01 ± 0.54	1.63 ± 0.45
	1000	**5.16 ± 1.59****	**25.21 ± 2.49****	**39.81 ± 2.23****	**60.53 ± 2.81****
	2000	**16.88 ± 3.04****	**94.11 ± 0.71****	**82.22 ± 2.41****	**61.05 ± 3.95****
lauric acid	0	0.95 ± 0.42	2.22 ± 1.11	0.88 ± 0.50	1.96 ± 0.54
	100	1.22 ± 0.28	2.46 ± 0.67	2.66 ± 0.59	2.60 ± 0.59
	1000	**52.27 ± 2.56****	**72.70 ± 4.61****	**15.78 ± 1.08****	**38.00 ± 2.44****
	2000	**46.94 ± 3.70****	**63.92 ± 6.25****	**21.27 ± 3.72****	**41.28 ± 3.22****
myristic acid	0	1.79 ± 0.52	2.51 ± 0.66	0.88 ± 0.50	1.96 ± 0.54
	100	2.81 ± 0.86	3.02 ± 0.54	1.31 ± 0.07	1.57 ± 0.52
	1000	5.16 ± 1.59	6.36 ± 1.32	**2.46 ± 0.26****	2.11 ± 0.37
	2000	**16.88 ± 3.04****	6.36 ± 1.98	**2.29 ± 0.35****	3.76 ± 0.65
palmitic acid	0	2.59 ± 0.55	3.80 ± 1.16	2.23 ± 0.42	2.61 ± 0.26
	100	4.88 ± 1.36	3.86 ± 0.93	3.33 ± 0.70	3.03 ± 0.68
	1000	8.37 ± 2.49	6.78 ± 0.91	3.36 ± 0.77	**5.44 ± 0.65***
	2000	12.78 ± 6.16	**8.27 ± 1.28***	**6.71 ± 1.16***	**4.91 ± 0.69***
oleic acid	0	2.59 ± 0.55	3.80 ± 1.16	2.23 ± 0.42	2.61 ± 0.26
	100	3.54 ± 0.63	5.01 ± 1.00	3.70 ± 0.81	2.42 ± 0.31
	1000	6.20 ± 2.05	6.55 ± 1.64	3.54 ± 0.88	**4.49 ± 0.52***
	2000	6.68 ± 11.72	7.48 ± 1.26	**4.52 ± 0.42***	**4.58 ± 0.74***
linoleic acid	0	2.59 ± 0.55	3.80 ± 1.16	2.23 ± 0.42	2.61 ± 0.26
	100	4.62 ± 0.60	3.79 ± 1.15	3.12 ± 0.43	3.30 ± 0.61
	1000	5.34 ± 1.85	4.96 ± 1.03	3.72 ± 0.50	3.41 ± 0.48
	2000	6.26 ± 1.19	6.17 ± 0.81	**4.72 ± 1.03***	**5.10 ± 0.56****

[a]The concentrations were at the level of 0, 100, 1000, and 2000 μmol/L, 12 and 24 h after inoculation (Trials 1 and 2). Data are shown as means ± SE with six replicates. Means were compared between control and each fatty acid treatment in the column using Dunnett's test. *$P < 0.05$, **$P < 0.01$.

oils (i.e., soybean oil),[36] farmers could harness the nematicidal activity of the seed cakes. In addition, both FAs have also been detected in gutter oil,[19] which is abundant in China causing challenging disposal problems. With the vast amount of available seed cakes and gutter oil, the characteristic antagonistic effects of palmitic acid (C16:0) and oleic acid (C18:1Δ^{9c}) against *M. incognita* may open the door for efficient exploitation of these resources for the control of *M. incognita*.

Apart from the direct inhibitory effects of FAs on RKNs, the induced plant resistance associated with the addition of FAs might partially contribute to the suppression of RKNs. For example, jasmonic acid is derived from FAs and is involved in the plant defense response to the nematode attack.[37] The nematicidal properties of β-amino-butyric acid against the root-knot nematode (*M. javanica*) were also related to the induced systemic resistance.[31] The addition of pure FAs, seed cakes, or other organic amendments might promote natural enemies and cause the top-down regulation of plant parasitic nematodes.[38] Moreover, the strong inhibitory effect of fatty acids on phytopathogenic fungi, such as capric acid, might indirectly enhance the plant vigor.[2] All of these hypotheses need to be verified in future studies.

In addition, other odd-carbon FAs [e.g., propionic acid (C3:0),[39] enanthic acid(C7:0),[34] pelargonic acid(C9:0),[34] and undecylic acid(C11:0)][34] and FA derivatives, such as pelargonic acid esters (C9:0),[7,34] might also have the potential to be used as effective nematicides.

There was a significant difference in nematicidal activity of fatty acids against the pine wood nematode with respect to the carbon chain length, with strong nematicidal activity being associated with the C8−C11 fatty acids.[34] In agreement with them, capric (C10:0) showed the best performance on nematode suppression in this study. Therefore, the carbon chain length should be considered in determining and utilizing the nematicidal properties of fatty acids and their derivatives.

Conclusions. Using natural substances as alternatives to synthetic chemical pesticides is attractive because they are less recalcitrant in the environment, with fewer nontargeted, toxic

effects than traditional agrochemicals.[40] The tested fatty acids in this study are natural fatty acids so they possess the above attributes and can be used in effective management of pest nematodes without causing environmental problems due to their nontoxic activities and environmental friendliness. Further research interest may focus on evaluating the effects of fatty acids on other plant parasitic nematodes, especially with the field trials. Meanwhile, information is also needed about direct application methods and appropriate schedules for FAs to maintain the optimal nematicidal activity with minimal side-effects on plant vigor. In addition, more studies are required to evaluate the mode of action of a combination of FAs against plant parasitic nematodes for management practices.

AUTHOR INFORMATION

Corresponding Author

*Email: ruanweibin2004@hotmail.com. Tel: 86-22-23504397 .

Funding

This research was jointly supported by the Special Fund for Agro-Scientific Research of the Public Interest of P. R. China (201103018) and the National Natural Science Foundation of China (31170412).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We gratefully acknowledge J. Zhu and J.-g. Wang for valuable comments on the manuscript.

REFERENCES

(1) Altieri, C.; Bevilacqua, A.; Cardillo, D.; Sinigaglia, M. Antifungal activity of fatty acids and theirmonoglycerides against *Fusarium* spp. in a laboratory medium. *Int. J. Food Sci. Technol.* **2009**, *44*, 242−245.

(2) Liu, S. Y.; Ruan, W. B.; Li, J.; Xu, H.; Wang, J. G.; Gao, Y. B. Biological control of phytopathogenic fungi by fatty acids. *Mycopathologia* **2008**, *166*, 93−102.

(3) Walters, D. R.; Walker, R. L.; Walker, K. C. Lauric acid exhibits antifungal activity against plant pathogenic fungi. *J. Phytopathol.* **2003**, *151*, 228−230.

(4) Amruthesh, K. N.; Geetha, N. P.; Lyngs Jørgensen, H. J.; de Neergaard, E.; Shekar Shetty, H. Unsaturated fatty acids from zoospores of *Sclerospora graminicola* induce resistance in pearl millet. *Eur. J. Plant Pathol.* **2005**, *111*, 125−137.

(5) Djian, C.; Ponchet, M.; Cayrol, J. C. Nematocidal properties of carboxylic-acids and derivatives. *Pestic. Biochem. Physiol.* **1994**, *50*, 229−239.

(6) Schwarz, M.; Köpcke, B.; Weber, R. W. S.; Sterner, O.; Anke, H. 3-Hydroxypropionic acid as a nematicidal principle in endophytic fungi. *Phytochemistry* **2004**, *65*, 2239−2245.

(7) Davis, E. L.; Meyers, D. M.; Dullum, C. J.; Feitelson, J. S. Nematicidal activity of fatty acid esters on soybean cyst and root-knot nematodes. *J. Nematol.* **1997**, *29* (2S), 677−84.

(8) Ntalli, N. G.; Menkissoglu-Spiroudi, U.; Giannakou, I. Nematicidal activity of powder and extracts of *Melia azedarach* fruits against *Meloidogyne incognita*. *Ann. Appl. Biol.* **2010**, *156*, 309−317.

(9) Ntalli, N. G.; Vargiu, S.; Menkissoglu-Spiroudi, U.; Caboni, P. Nematicidal carboxylic acids and aldehydes from *Melia azedarach* fruits. *J. Agric. Food Chem.* **2010**, *58*, 11390−11394.

(10) Mahran, A.; Tenuta, M.; Hanson, M. L.; Daayf, F. Mortality of *Pratylenchus penetrans* by volatile fatty acids from liquid hog manure. *J. Nematol.* **2008**, *40*, 119−126.

(11) Xiao, J. L.; Zhu, J.; Chen, S. Y.; Ruan, W. B.; Miller, C. A novel use of anaerobically digested liquid swine manure to potentially control soybean cyst nematode. *J. Environ. Sci. Health, Part B* **2007**, *42*, 749−757.

(12) Yang, L. L.; Huang, Y.; Liu, J.; Ma, L.; Mo, M. H.; Li, W. J.; Yang, F. X. *Lysinibacillus mangiferahumi* sp. nov., a new bacterium producing nematicidal volatiles. *Antonie van Leeuwenhoek* **2012**, *102*, 53−59.

(13) Christopoulou, E.; Lazaraki, M.; Komaitis, M.; Kaselimis, K. Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. *Food Chem.* **2004**, *84*, 463−474.

(14) Ogunniyi, D. S. Castor oil: A vital industrial raw material. *Bioresour. Technol.* **2006**, *97*, 1086−1091.

(15) Zdunczyk, Z.; Minakowski, D.; Frejnagel, S.; Flis, M. Comparative study of the chemical composition and nutritional value of pumpkin seed cake, soybean meal and casein. *Nahrung* **1999**, *43*, 392−395.

(16) Izquierdo, N. G.; Aguirrezábal, L. A. N.; Andrade, F. H.; Geroudet, C.; Valentinuz, O.; Pereyra Iraola, M. Intercepted solar radiation affects oil fatty acid composition in crop species. *Field Crops Research* **2009**, *114*, 66−74.

(17) Parashar, A.; Sinha, N.; Singh, P. Lipid contents and fatty acids composition of seed oil from twenty five pomegranates varieties grown in India. *Adv. J. Food Sci. Technol.* **2010**, *2*, 12−15.

(18) Tiyagi, S. A.; Alam, M. M. Efficacy of oil-seed cakes against plant-parasitic nematodes and soil-inhabiting fungi on mungbean and chickpea. *Bioresour. Technol.* **1995**, *51*, 233−239.

(19) Wu, H. Q.; Huang, X. L.; Chen, J. H.; Zhu, Z. X.; Lin, X. S.; Huang, F.; Ma, Y. F.; Luo, H. T.; Deng, X. A Novel Method for the Identification of Gutter Oils Using SPME/GC-MS. *J. Instrum. Anal.* **2012**, *31*, 1−6.

(20) Atkinson, H. J.; Lilley, C. J.; Urwin, P. E. Strategies for transgenic nematode control in developed and developing world crops. *Curr. Opin. Biotechnol.* **2012**, *23*, 251−256.

(21) Sasser, J. N.; Eisenback, J. D.; Carter, C. C.; Triantaphyllou, A. C. The international *Meloidogyne* project: Its goals and accomplishments. *Annu. Rev. Phytopathol.* **1983**, *21*, 271−288.

(22) Rich, J. R.; Dunn, R. A.; Noling, J. W. Nematicides: Past and present uses. *Nematology* **2004**, *21*, 1179−1200.

(23) Jothi, G.; Babu, R. S.; Ramakrishnan, S.; Rajendran, G. Management of root lesion nematode, *Pratylenchus delattrei* in crossandra using oil cakes. *Bioresour. Technol.* **2004**, *93*, 257−259.

(24) Abdel-Rahman, F. H.; Clark, S.; Saleh, M. A. Natural organic compounds as alternative to methyl bromide for nematodes control. *J. Environ. Sci. Health, Part B* **2008**, *43*, 680−685.

(25) Akhtar, M. Nematicidal potential of the neem tree *Azadirachta indica* (A. Juss). *Integrated Pest Management Reviews* **2000**, *5*, 57−66.

(26) Mian, I. H.; Rodriguez-Kabana, R. Soil amendments with oil cakes and chicken litter for control of *Meloidogyne arenaria*. *Nematropica* **1982**, *12*, 205−220.

(27) Hussey, R. S.; Barker, K. R. A comparison of methods of collecting inocula of *Meloidogyne spp*. including a new technique. *Plant Dis. Rep.* **1973**, *57*, 1025−1028.

(28) Xiao, J. L.; Chen, S. Y.; Zhu, J.; Ruan, W. B. Effect of liquid swine manure on hatch and viability of *Heterodera glycines*. *J. Nematol.* **2008**, *40* (2), 152−160.

(29) Chen, S. Y.; Dickson, D. W. A technique for detecting viability of second-stage juvenile of *Heterodera glycines*. *J. Nematol.* **2000**, *32*, 117−121.

(30) Browning, M.; Wallace, D. B.; Dawson, C.; Alm, S. R.; Amador, J. A. Potential of butyric acid for control of soil-borne fungal pathogens and nematodes affecting strawberries. *Soil Biol. Biochem.* **2006**, *38*, 401−404.

(31) Mitkowski, N. A.; Jordan, K. S. Organic acids as preplant nematicides for use against *Meloidogyne hapla* and *Pratylenchus penetrans*. *Phytopathology* **2003**, *93*, S62.

(32) Sahebani, N.; Hadavi, N. S.; Zade, F. O. The effects of beta-amino-butyric acid on resistance of cucumber against root-knot nematode, *Meloidogyne javanica*. *Acta Physiol. Plant.* **2011**, *33*, 443−450.

(33) Barbosa, L. C. A.; Barcelos, F. F.; Demuner, A. J.; Santos, M. A. Investigation-Research: Chemical constituents from *Mucuna aterrima*

with activity against *Meloidogyne incognita* and *Heterodera glycines*. *Nematropica* **1999**, *29*, 81−88.
(34) Seo, S. M.; Kim, J.; Kim, E.; Park, H. M.; Kim, Y. J.; Park, I. K. Structure-activity relationship of aliphatic compounds for nematicidal activity against pine wood nematode (*Bursaphelenchus xylophilus*). *J. Agric. Food Chem.* **2010**, *58*, 1823−1827.
(35) Ruan, W. B.; Wang, J.; Pan, H.; Li, H. B.; Wang, J. G.; Zhang, F. S.; Gao, Y. B. Effects of sesame seed cake allelochemicals on the growth cucumber (*Cucumis sativus* L. cv. Jinchun 4). *Allelopathy Journal* **2005**, *16*, 217−225.
(36) Lichtenstein, A. H.; Matthan, N. R.; Jalbert, S. M.; Resteghini, N. A.; Schaefer, E. J.; Ausman, L. M. Novel soybean oils with different fatty acid profiles alter cardiovascular disease risk factors in moderately hyperlipidemic subjects. *Am. J. Clin. Nutr.* **2006**, *84*, 497−504.
(37) Nahar, K.; Kyndt, T.; De Vleesschauwer, D.; Höfte, M.; Gheysen, G. The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. *Plant physiol.* **2011**, *157*, 305−316.
(38) Chavarria-Carvajal, J. A.; Rodriguez-Kabana, R. Changes in soil enzymatic activity and control of *Meloidogyne incognita* using four organic amendments. *Nematropica* **1998**, *28*, 7−18.
(39) McElderry, C. F.; Browning, M.; Amador, J. A. Effect of short-chain fatty acids and soil atmosphere on *Tylenchorhynchus spp*. *J. Nematol.* **2005**, *37*, 71−77.
(40) Ferris, H.; Bongers, T.; De Goede, R. G. M. A framework for soil food web diagnostics: Extension of the nematode faunal analysis concept. *Appl. Soil Ecol.* **2001**, *18*, 13−29.