JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Nematicidal Activity of 2-Thiophenecarboxaldehyde and Methylisothiocyanate from Caper (*Capparis spinosa*) against *Meloidogyne incognita*

Pierluigi Caboni,*[,†] Giorgia Sarais,[†] Nadhem Aissani,[†] Graziella Tocco,[†] Nicola Sasanelli,[§] Barbara Liori,[†] Annarosa Carta,[‡] and Alberto Angioni[†]

[†]Department of Life and Environmental Science and [‡]Dipartimento di Scienze Biomediche, Università degli Studi di Cagliari, Via Ospedale 72, 09124 Cagliari, Italy

[§]Institute for Plant Protection, UOS of Bari, Consiglio Nazionale delle Ricerche, Via G. Amendola, 122/D 70126 Bari, Italy

ABSTRACT: New pesticides based on plant extracts have recently gained interest in the development of nontoxic crop protection chemicals. Numerous research studies are focused on the isolation and identification of new active compounds derived from plants. In this manuscript we report about the use of the Mediterranean species *Capparis spinosa* as a potent natural nematicidal agent against the root knot nematodes *Meloidogyne incognita*. Leaves, stems, and caper buds of *Capparis spinosa* were used to obtain their methanol extracts (LME, SME, BME) that were successively *in vitro* tested against second stage nematode juveniles (J2). In terms of paralysis induction, the methanol extract of the stem part (SME) was found more effective against *M. incognita* and then the caper methanol buds and leaves extracts. The chemical composition analysis of the extracts carried out by GC/MS and LC/MS techniques showed that methylisothiocyanate was the main compound of SME. The EC_{50} for SME after 3 days of immersion was 215 ± 36 mg/L. The constituent components of SME such as 2-thiophenecarboxaldehyde and methylisothiocyanate were successively in vitro tested for their nematicidal activity against J2. Both compounds induced paralysis on root knot nematodes ranking first (EC_{50} = 7.9 ± 1.6, and 14.1 ± 1.9 mg/L respectively) for *M. incognita*. Moreover, 2-thiophenecarboxaldehyde showed a strong fumigant activity.

KEYWORDS: *root-knot nematodes, bionematicides, biopesticides, alternative nematode control methods, fumigants*

INTRODUCTION

Plant secondary metabolites are a subset of a secondary plant metabolite produced by an individual of one species that affects the survival, health, growth, and reproduction or population biology of another species.[1] Allelochemicals can occur in plants as volatile deterrents and repellents, inhibitors of oviposition, or nonvolatile antifeedant.[2,3] We recently discovered that volatile plant secondary metabolites such (*E*,*E*)-2,4-decadienal and (*E*)-2-decenal from *Ailanthus altissima* (Mill.), 2-undecanone from *Ruta chalepensis* L., and furfural from *Melia azedarach* L. were active, against root knot nematodes *Meloydogyne incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Chitw,[4−7] supporting the concept that unique combinations of plant volatiles are produced in response to attack by different pest species.

Many scientific studies reported data on the biological activity of plant extracts and their constituents against root-knot nematodes.[8−11] However, to date no studies have been published on the nematicidal effects of extracts of capers (*Capparis spinosa* L., *Capparaceae*) and its constituents against the root-knot nematode *M. incognita* except for Al-Banna, who found the root methanol extract to be not active against *M. incognita* and *M. javanica* at 20 mg/L.[12]

Caper is widely distributed in the Mediterranean area and West Asia, and it is used as a culinary spice as well as an anti-inflammatory agent in folk medicine, to treat earache and cough, and to expel intestinal worms.[13,14] The caper bush, known for the edible flower buds (capers), is a perennial winter-deciduous plant with large white flowers. Both buds and the fruit (caper berry) are usually consumed pickled.

Different chemical classes such as alkaloids, lipids, indoles, flavonoids, and aliphatic glucosinolates were identified in caper.[15] Aliphatic glucosinolates, belonging to sulfur secondary metabolites group, are hydrolyzed by the vacuolar enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) to produce isothiocyanates, nitriles, and thiocyanates in addition to glucose and sulfate ion. Enzymatic degradation of glucocapparin, a modified sugar found in capers,[16] produces the volatile allelochemical methylisothiocyanate. It is the major degradation product that imparts soil fumigant activity to both commercially available nematicides metham sodium and dazomet.[17] Methylisothiocyanate is also responsible for the biological activity of *Boscia senegalensis* (Pers) Lam. ex Poiret (*Capparaceae*) fruits and leaf extracts against stored grain insects.[18] Serra et al. reported that 2-phenyethylglucosinolate and its hydrolysis derivatives from water cress (*Nasturtium officinale* L.) were active against the potato cyst nematode *Globodera rostochiensis* (Woll.).[19] Interestingly, the suppressive nematicidal effect of soil-incorporated Brassicaceae plant parts containing glucosinolates has been known since 1951,[20] and today it is known as "biofumigation". Nowadays, different plant

Received: May 11, 2012
Revised: July 5, 2012
Accepted: July 6, 2012
Published: July 6, 2012

species are commonly used.[21] Nevertheless, to the best of our knowledge, the nematicidal effects of the caper plant extracts or its specific ingredients against *M. incognita* has not yet been investigated.

Many plant extracts active against nematodes have been reported in the literature. Unfortunately, every single compound present in these complex mixtures may contribute to both the beneficial and the adverse effects of the extract itself. This is why knowing the chemical composition of the extract is fundamental for better comprehension of its mode of action.

In the present investigation we report (1) the GC/MS and LC/MS chemical characterization of the aerial parts and caper buds of *C. spinosa* methanol extracts (SME, LME, BME), (2) the study of the in vitro paralyzing activity (EC_{50}) of the methanol extracts against *M. incognita* second-stage juveniles, and (3) the in vitro biological activity against the root-knot nematodes of pure compounds found in the extracts. In addition, we synthesized and tested in vitro some 2-thiophene carboxaldehyde derivatives which share with the parent compound the same aromatic scaffold but support oxime or nitrile as functional groups.

MATERIALS AND METHODS

Chemicals. Methylisothiocyanate, 2-naphtaldehyde, 2-thiophenecarboxaldehyde, 2.5-thiophenedicarboxyaldehyde, 2-pyridine carboxaldehyde, indole-3-carbonitrile, indole-3-carboxaldehyde, 1*H*-indole-2,3-dione, pyrrole-2-carboxaldehyde, 2-pyrrolidone, *N*-methyl-2-pyrrolecarboxaldehyde, methanol, Tween, and DMSO were purchased from Sigma Aldrich (Milan, Italy) with a purity >98−99%. Water was distilled through a Milli-Q apparatus.

Experimental Chemistry. Reaction progress was monitored by TLC using Aldrich silica gel 60 F254 (0.25 mm) plates. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 MHz spectrometer. IR spectra were recorded in Nujol mull or neat on a Perkin-Elmer 1310 spectrophotometer. Melting points were determined on a Stuart Scientific SMP 11 melting point apparatus.

Synthesis of 2,5-Thiophenedicarbonitrile. A mixture of 2,5-thiophenedicarboxaldehyde (2.14 mmol, 0.30 g) and $NH_2OH{\cdot}HCl$ (6.42 mmol, 0.45 g) in pyridine (22.26 mmol, 1.8 mL) was stirred at 100 °C for 2.5 h. After reaction, the solvent was removed under vacuum to give the crude products as a brown viscous liquid. Yield (%) = 55. IR (Nujol mull): 3280, 2320 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 7.65 (s, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 145.11, 119.67, 110.34 ppm.

Synthesis of 5-Formylthiophene-2-carbonitrile. A mixture of 2,5-thiophenedicarboxaldehyde (2.14 mmol, 0.30 g) and $NH_2OH{\cdot}HCl$ (3.21 mmol, 0.22 g) in pyridine (18.58 mmol, 1.5 mL) was stirred at 100 °C for 2.5 h. After reaction, solvent was removed under vacuum to give the crude products as an orange solid, which was recrystallized from ethanol. Yield (%) = 96. Mp = 198−200 °C. IR (Nujol mull): 3300, 2780, 2365, 1675 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.08 (s, 1H), 7.87 (d, 1H, J = 4.0 Hz), 7.43 (d, 1H, J = 4.0 Hz) ppm. ^{13}C NMR (500 MHz, DMSO): δ 182.11, 154.78, 139.54, 134.89, 120.00, 111.18 ppm.

Synthesis of 2-Thiophenecarboxaldehyde oxime. A mixture of 2-thiophene carboxaldehyde (8.93 mmol, 1 g) and hydroxylamine chlorhydrate (13.4 mmol, 0.93 g) was stirred at 100 °C for 1 h. After reaction, 15 mL of distilled water was added and the reaction mixture was extracted with ethyl acetate (3 × 15 mL). The organic phase was dried over sodium sulfate, and the solvent was removed under vacuum to give the product as a viscous pale green oil. Yield (%) = 62. IR (Nujol mull): 3270, 2570, 1635 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 9.04 (bs, 1H), 7.54 (d, 1H, J = 4.5 Hz), 7.22 (d, 1H, J = 5.3 Hz), 7.12 (m, 1H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 153.12, 144.12, 132.76, 122.67, 117.80 ppm.

Synthesis Thiophene-2-carbonitrile (F). A mixture of 2-thiophenecarboxaldehyde oxime (1.97 mmol, 0.25 g), methanesulfonyl chloride (1.97 mmol, 0.155 mL), and dry acid alumina was heated in an oil bath at 100 °C for 1 h. After reaction, 15 mL of ethyl acetate was added and the resulting mixture was filtered to remove alumina. The filtrate was washed with distilled water (2 × 10 mL), and the organic phase, previously dried on sodium sulfate, was concentrated to give the crude product as an oil. The pure product, as a yellow oil, was obtained after flash chromatography on silica gel (eluent dichloromethane). Yield (%) = 61. IR (neat): 3250, 2780, 2374 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.08 (s, 1H), 7.87 (d, 1H, J = 4.0 Hz), 7.43 (d, 1H, J = 4.0 Hz) ppm. ^{13}C NMR (500 MHz, DMSO): δ 182.11, 154.78, 139.54, 134.89, 120.00, 111.18 ppm.

Synthesis of 2-Pyridinecarboxaldehyde Oxime. A mixture of 2-pyridinecarboxaldehyde (9.34 mmol, 1 g) and hydroxylamine chlorhydrate (14 mmol, 0.97 g) was stirred at 100 °C for 1 h. After reaction, 15 mL of distilled water was added; the reaction mixture was neutralized and then extracted with ethyl acetate (3 × 15 mL). The organic phase was dried over sodium sulfate, and the solvent was removed under vacuum to give the product as a viscous pale yellow solid. Yield (%) = 70. Mp = 110−112 °C. IR (Nujol mull): 3280, 2550, 1645 cm^{-1}. ^{1}H NMR (500 MHz, CDCl3): δ 9.47 (bs, 1H), 8.64 (d, 1H, J = 4.5 Hz), 8.32 (s, 1H), 7.83 (d, 1H, J = 7.5 Hz), 7.72 (m, 1H), 7.29 (m, 1H) ppm. ^{13}C NMR (500 MHz, CDCl3): δ 152.51, 149.04, 144.20, 136.12, 127.00, 123.73 ppm.

Synthesis of Pyridine-2-carbonitrile. A mixture of 2-pyridinecarbaldehyde oxime (2.46 mmol, 0.30 g), methanesulfonyl chloride (2.46 mmol, 0.19 mL), and dry acid alumina was heated in an oil bath at 100 °C for 1 h. After reaction, 15 mL of ethyl acetate/acetonitrile (1/1) was added and the resulting mixture was filtered to remove alumina. The filtrate was washed with distilled water (2 × 10 mL), and the organic phase, previously dried on sodium sulfate, was concentrated to give the crude product as an oil. The pure product, as a yellow oil, was obtained after flash chromatography on silica gel (eluent hexane/ethyl acetate 1/1). Yield (%) = 67. IR (neat): 3690, 2235 cm^{-1}. ^{1}H NMR (500 MHz, CDCl3): δ 7.75 (m, 2H), 7.22 (d, 1H, J = 4.5 Hz) ppm. ^{13}C NMR (500 MHz, $CDCl_3$): δ 137.32, 132.60, 127.45, 114.21, 109.91 ppm.

Plant Materials and Chemicals. The aerial parts and buds of *C. spinosa* were collected in Cagliari, Sardinia, in Feb 2012. Voucher specimens (CaboniCS050) were deposited in the Department of Life Science and Environment for species identification. Fresh aerial plant parts (100 g) were ground and extracted with methanol (1:1 w/v) in a sonicator apparatus for 15 min, filtered through a Whatman no. 40 filter, and centrifuged for 15 min at 13 000 rpm. The extract was then analyzed and identified by GC/MS. Plant materials were also dried at 105 °C for 24 h. The moisture of the stems, leaves, and buds were 51.18%, 79.12%, and 79.60% respectively.

GC/MS Analysis. Chromatographic separation and identification of the main components of methanol extracts of *Capparis spinosa* was performed on a Trace GC Ultra Gas Chromatograph (Thermo Finnigan, MA) coupled with a Trace DSQ mass spectrometry detector, a split−splitless injector, and an Xcalibur MS platform. The column was a CP-WAX 57CB from Varian (60 m long, 0.25 mm i.d., and 0.25 μm film thickness; Varian Inc., USA). The injector and transfer line were at 200 °C. The oven temperature was programmed as follows: 50 °C (hold 1 min) then raised to 220 °C (3 °C/min) and isothermally hold for 13 min. Helium was the carrier gas at a constant flow rate of 1 mL/min; 1 μL of each sample was injected in the splitless mode (60 s). Mass spectrometry acquisition was carried out using the continuous (EI positive) scanning mode from 45 to 500 amu. *C. spinosa* methanol extract components were identified by (a) comparison of their relative retention times and mass fragmentation with those of authentic standards and (b) computer matching against a NIST/EPA/NIH Mass Spectral Library (NIST 08). For stems, leaves, and buds quantitative analysis of methylisothiocyanate and 2-thiophenecarboxaldehyde were carried out in triplicate with the external standard method .

J2 Paralysis Bioassays. An Italian population of *M. incognita* was reared on tomato (*Solanum lycopersicum* Mill.) cv. Roma VF for 2

months in a glasshouse at 25 ± 2 °C. Batches of 30 egg masses (averaging 4500 eggs/batch) were collected from infected tomato roots. Batches were placed on 2 cm diameter sieves (215 μm aperture) in a 3.5 cm diameter Petri dish, and then 3 mL of distilled water was added as a natural hatching agent according to an already described methodology.[22] Batches were then incubated in a growth cabinet at 25 ± 2 °C in dark condition. Emerging juveniles J2 (24 h) were removed and collected every day and used for the experiments (Figure 1).

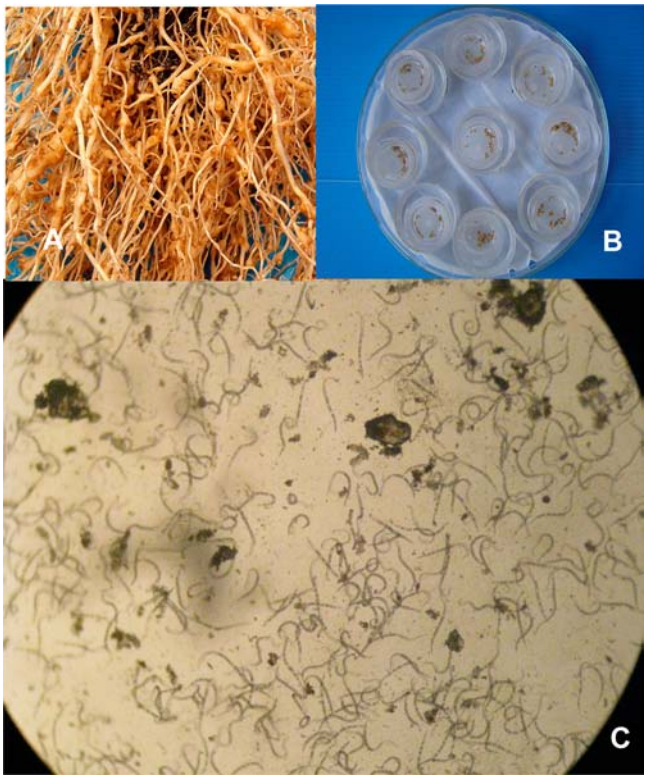

Figure 1. *M. incognita* infected tomato roots with galls and egg masses (A), batches with egg masses (B), and hatched juveniles (C).

Nemathorin 150EC (a.i. fosthiazate 15%, Hellafarm Co), distilled water, as well as solvent carriers served as controls for paralysis correction. The LME, SME, and BME were tested against root-knot nematodes at a dose range of 10−5.000 mg/L and assessed for toxicity at 72 h.

Methylisothiocyanate and 2-thiophenecarboxaldehyde found in stem and leaves methanol extracts were tested against J2 at a dose range of 1.0−1000 mg/L. As part of a study on the nematicidal activity we tested on nematodes structurally related aromatic heterocyclic aldehydes, oximes, nitriles, and indoles such as 2,5-thiophenenedicarboxaldehyde, 2,5-thiophenedinitrile, 5-formylthiophene-2-carbonitrile, 2-pyridinecarboxaldehyde, 3-pyridinecarboxaldehyde, 2-pyridinecarbaldehyde oxime, 2-pyridincarbonitrile, 2-naphthaldhehyde, 2-thiophenecarboxaldehyde oxime, 2-thiophenecarbonitrile, indole-3-carbonitrile, indole-3-carboxaldehyde, 1*H*-indole-2,3-dione, pyrrole-2-carboxaldehyde, 2-pyrrolidone, and *N*-methyl-2-pyrrolecarboxaldehyde (Figure 2). All pure compounds were subjected to dose−response experiments against J2 at different dose−response ranges. Stock solutions of pure compounds were prepared in methanol to overcome insolubility, whereas Tween 20 in twice distilled water was used for further dilution. Final concentration of methanol in each well never exceeds 1% (v/v) because preliminary experiments showed that this concentration was not toxic to nematodes. Distilled water as well as a mixture of water and Tween (0.3% v/v) (carrier control) served as controls. In all cases working solutions were prepared containing double the test concentration and mixed in CellstarR 96-well cell culture plates (Greiner Bio-One) at a ratio of 1:1 (v/v) with suspension of 25 J2 added to each well. Multiwell plates were covered to avoid evaporation and maintained in the dark at 20 °C. Juveniles were observed with the aid of an inverted microscope (Zeiss, 3951, Germany) at 10× and 20× after 1 and 24 h for pure compounds and 72 h for methanolic extracts of *C. spinosa* were ranked in two distinct categories: motile or paralyzed/death. Moreover, at that point nematodes were moved to plain water after washing in tap water through a 20 μm pore sieve to remove the excess of extracts. Numbers of motile and paralyzed or deaths J2 were assessed by stimulating the juvenile body with a needle and then counting them. Paralysis experiments were performed four times, and every treatment was replicated six times.

Fumigant Activity Against J2. Nematode juveniles were immersed in distilled water in wells adjacent to the treatment wells where the test solution (LME, SME, BME, and test compounds) was poured. For every treatment well four adjacent ones were used, and in each well only one treatment dose was used. Paralysis or death percentages recording the fumigant activity in the four adjacent to the treatment wells served as an experiment's treatment replication with 50 J2 instead of 25. Assessments were made 1 h (1 h) and 1 day (1 d) after experiments were started.

Statistical Analysis. Since paralysis in solvent (methanol, Tween-20) did not differ significantly from that observed in distilled water, the percentages of paralyzed/death J2 were corrected by eliminating the natural death/paralysis in the water control (0−5% of total number of J2) according to the Schneider Orelli's formula:[22] corrected % = {((mortality % in treatment) − (mortality % in control))/(100 − (mortality % in control))} × 100, and they were analyzed (ANOVA) after being combined over time. Since ANOVA indicated no significant treatment by time interaction, means were averaged over experiments. Corrected percentages of paralyzed/death J2 treated with tested compounds were subjected to nonlinear regression analysis using the log−logistic equation proposed by Seefeldt et al. in 1995:[23] $Y = C + (D - C)/\{1 + \exp[b(\log(x) - \log(EC_{50}))]\}$, where C = the lower limit, D = the upper limit, b = the slope at the EC_{50}, and EC_{50} = the test substances' concentration required for 50% death/paralysis of nematodes after eliminating the control (natural death/paralysis). In the regression equation, the test substances' concentration was the independent variable (x) and the paralyzed J2 (percentage increase over water control) was the dependent variable (y). The mean value of the six replicates per test substances concentration and immersion (or exposure to vapors) period was used to calculate the EC_{50} value. Mean data values were reported with the respective standard deviations.

RESULTS AND DISCUSSION

Gas chromatography coupled with mass spectrometry is the elective technique for chemical characterization of plant extracts because it allows identification of a large number of plant metabolites. As previously reported,[3] we developed a method for direct injection without derivatization of plant methanol extracts. By use of a Carbowax column CP-WAX 57CB we were able to separate polar and medium polar plant secondary metabolites from *C. spinosa* plant methanol extracts (SME, LME, BME), such as methylisothiocyanate, 5-hydroxymethyl-2-furancarboxaldehyde, furfural, and 2-thiophenecarboxaldehyde (Tables 1− 3). We also tried to determine the chemical composition of the methanol extracts by liquid chromatography−mass spectrometry−time-of-flight (LCMS-TOF), but isothiocyanates or aldehydes were not detected because of their volatility and low ionization efficiency during atmospheric pressure ionization.

As a result of the GC/MS analysis, we were able to identify 24 plant metabolites of the methanol extract of stem (Table 1). We confirmed by cochromatography the presence of 2-thiophenecarboxaldehyde in the SME, and to our knowledge, this is the first report of this compound found in a plant extract. We found 2-thiophenecarboxaldehyde in stem extract at a

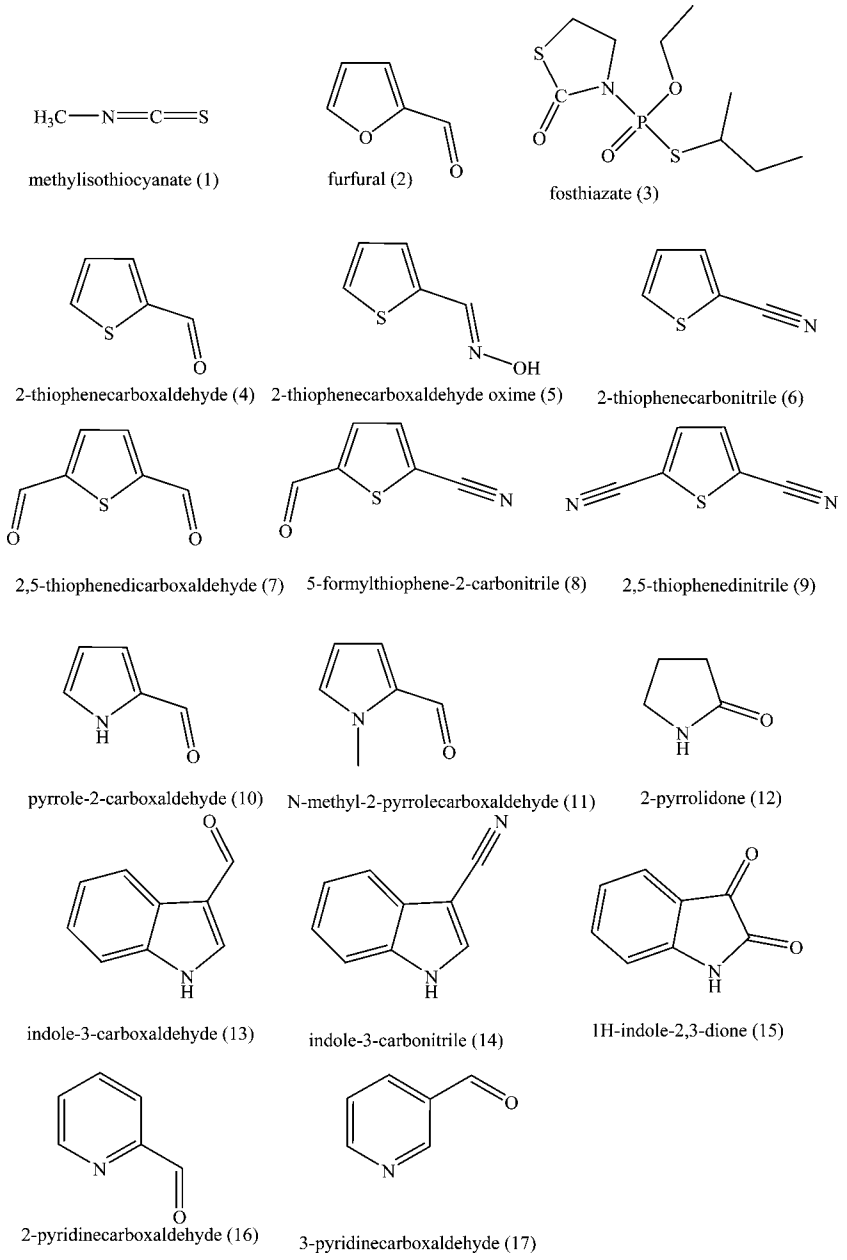

Figure 2. Chemical structures of tested compounds.

concentration of 1.12 ± 0.31 mg/kg, while methylisothiocyanate levels in stem, leaves, and buds were 116 ± 42, 7.33 ± 3.78, and 3.87 ± 0.98 mg/kg on a dry weight basis, respectively.

Without taking into account plant compound bioavailability or the synergetic effect when SME was tested against *M. incognita*, a dose−response relationship was established and significant paralysis/death of J2 was evident after 3 days of exposure with an $EC_{50/3d}$ value calculated at 215 mg/L (Table 2). This value is rather low considering the activities of *R. chalepensis* methanol extracts against *M. incognita*, exhibiting an EC_{50} value of 1001 mg/L after 1 day of J2 immersion in test solutions.[5] Under the same conditions extracts from leaves and buds extracts had no effect at all the tested concentrations (10−1000 mg/L).

Among secondary plant metabolites identified in the methanol extract of different parts of the plant *C. spinosa*, methylisothiocyanate, furfural, and 2-thiophenecarboxaldehyde were the most active compounds against J2 with $EC_{50/1d}$ of 7.86, 8.50, and 14.08, mg/L, respectively. Moreover, 2-pyridinecarboxaldehyde and 2-thiophenecarboxaldehyde showed a fumigant activity with an $EC_{50/1d}$ of 62 and 100 mg/L respectively. The activity of methylisothiocyanate and 2-thiophenecarboxaldehyde against *M. incognita* is rather high if compared to the nematicidal activity of fosthiazate ($EC_{50/1d}$ = 0.40 mg/L) (Table 3). This is the first report of the irreversible nematicidal activity of 2-thiophenecarboxaldehyde as a constituent of *C. spinosa* against *M. incognita*. According to our results, methylisothiocyanate was the principal nematicidal constituent of *C. spinosa* for all extracts (SME, LME, BME) with percent concentrations of 64.57%, 17.56%, and 2.13%, while 2-thiophenecarboxaldehyde was found only in the stem extract (SME) at a 0.19% concentration. This evidence supports the fact that leaves and buds extracts are not active in in vitro experiments against J2. Recently, we reported the

Table 1. GC/MS Analysis of the Methanolic Extract of Caper Stems (SME)

compound	R_t	relative %	mw	EIMS (m/z) (amu) (abundance)
methylisothiocyanate	16.17	64.57	73	73 (100); 72 (52); 70 (12); 45 (7); 58 (7)
methyl pyrazine	18.09	0.84	94	94 (100); 67 (45); 53 (15)
3-hydroxy 2-butanone	19.24	0.43	88	45 (100); 88 (30); 73 (10); 55 (5)
unknown	19.83	1.48		74 (100); 45 (18); 74 (10)
2,5-dimethyl pyrazine	20.47	0.05	108	108 (100); 72 (38)
2,3-dimethyl pyrazine	21.22	0.15	108	108 (100); 67 (90)
4-hydroxy-4-methyl-2-pentanone	22.04	1.88	116	59 (100); 101 (55)
o-methyl-*n*-methyl carbamate	24.90	3.93	89	58(100); 89(55); 74(42); 59(22)
furfural	25.92	3.62	96	96 (100); 95 (59); 67 (10)
2-furyl methyl ketone	27.60	0.16	110	95 (100); 110 (33)
5-methyl-2-furaldehyde	30.37	0.67	110	110 (100); 108 (88); 53 (45); 81 (10)
benzoic acid methyl ester	32.23	0.28	136	105 (100); 77 (48); 136 (47); 51 (15)
furfuryl alcohol	33.64	1.00	98	98 (100); 97 (52); 69 (30); 81 (28); 70 (24)
2-(1*H*-imidazol-5-yl)propan-1-amine	34.39	0.06	125	96 (100); 95 (83); 68 (30); 59 (22); 81 (10)
3-thiophenecarboxaldehyde	34.53	0.22	112	111 (100); 112 (95); 83 (20); 58 (10); 57 (9)
2-thiophenecarboxaldehyde	35.10	0.19	112	111 (100); 112 (75); 58 (11); 57 (8); 83 (7)
(5-methyl-2-furyl)methanol	35.94	0.18	112	95 (100); 112 (72); 97 (40); 69 (32); 53 (20)
unknown	36.69	0.07		138(100); 123(90); 95(35);63 (18)
unknown	37.40	4.13		59 (100); 60 (3); 93 (2)
β-damascenone	39.67	0.12	190	69 (100); 121 (60); 190 (30); 105 (28); 91(15); 77(12); 79(8)
unknown	42.30	3.45		105 (100); 74 (20); 58 (15); 60 (13); 72 (11); 75 (9); 61 (7)
unknown	43.60	0.53		71(100); 55 (68)
methyl 2-pyrrolyl ketone	44.98	0.32	109	94(100); 109(82); 66(55)
unknown	45.48	0.23		127(100); 55(13); 99 (10)
formic acid. allyl ester	51.62	1.16	86	57(100); 58(43)
4-acetoxy-3-methoxystyrene	52.27	0.22	192	150(100); 135(63); 107(30); 77(22)
2.3-dihydro-3.5-diidroxy-6-methyl 4*H*-piran-4-one	54.49	0.59	144	144(100); 101(65); 72(32); 55(28); 73(20)
thioacetamide	56.99	0.32	75	75(100); 59(68)
5-hydroxymethyl-2-furan carboxaldehyde	61.54	8.08	126	97(100); 126(52); 69(32)
2-thiophene ethanol	64.20	1.06	128	97(100); 128(50); 69(32)

Table 2. Nematicidal Activity of *C. spinosa* Extracts (SME, LME, and BME) against *M. incognita* Calculated after 1 and 3 Days of Immersion in Test Solutions[a]

extract	$EC_{50/1d}$ (mg/L ± SD)	$EC_{50/3d}$ (mg/L ± SD)
SME	>1000	215 ± 36
LME	>1000	>1000
BME	>1000	>1000

[a] *C. spinosa* methanolic extracts of stem, SME, leaves, LME, and buds, BME.

Table 3. EC_{50} Values (mg/L ± SD, n = 4) of Pure Compounds Against *M. incognita* at 1 h and 1 Day After Treatment

compound	1 h, EC_{50} (mg/L)	1 d, EC_{50} (mg/L)
methyl isothiocyanate	>10	7.86 ± 1.57
2,5-thiophenenedicarboxaldehyde	1.20 ± 0.15	1.13 ± 0,36
2,5-thiophenedinitrile	>250	>250
2-pyridinecarboxaldehyde[a]	25.0 ± 2.4	3.60 ± 1.13
3-pyridinecarboxaldehyde	>250	>10
2-thiophenenecarboxaldehyde[a]	105 ± 10	14.08 ± 1.86
2-thiophenecarboxaldehyde oxime	>250	>250
2-thiophenecarbonitrile	>100	>100
5-formylthiophene-2-carbonitrile	>250	>250
indole-3-carbonitrile	>250	68.9 ± 14.1
indole-3-carboxaldehyde	>250	301 ± 33
1*H*-indole-2,3-dione	>250	>250
pyrrole-2-carboxaldehyde	>1000	392 ± 32
2-pyrrolidone	>250	>250
N-methyl-2-pyrrolecarboxaldehyde	>1000	>1000
furfural	11.40 ± 1.25	8.50 ± 0.89
fosthiazate	3.30 ± 0.95	0.40 ± 0.30

[a] These compounds showed 100% mortality after 24 h of juveniles immersion in 100 mg/L solution.

strong nematicidal activity of furfural against *M. incognita* and *M. javanica*[6] present in extracts of buds and stem at percent concentrations of 1.12% and 3.62%, respectively.

Considering that aldehydes such as furfural and 2-thiophencarboxaldehyde were active against juveniles of *M. incognita*, we prepared structurally related compounds with the same aromatic scaffold but supporting selected functional groups such as oxime and nitriles. The in vitro experiments against J2 evidenced the interesting biological activity of 2,5-thiophenedicarboxaldehyde, 2-pyridincarboxaldehyde, 2-thiophenecarboxaldehyde, and indole-3-carbonitrile with EC_{50} of 1.13, 3.60, 14.08, and 68.9 mg/L, respectively (Table 3). Together with methylisothiocyanate and furfural, 2-thiophenecarbaxoaldehyde and 2,5-thiophenecarbaxoaldehyde were the most active compounds tested against J2 while their derivatives 5-formylthiophenecarbonitrile and 2-thiophenecarboaxaldehyde oxime were not active at the tested concentration. Interestingly, indole-3-carbonitrile was 4-fold more potent than indole-3-carboxaldehyde. Pyrrole 2-carboxaldehyde showed an $EC_{50/1d}$ of 392 mg/L, while its *N*-methyl derivative *N*-methyl-2-pyrrolecarboxaldehyde was inactive. Moreover, 2-pyridinecarboxaldehyde was very active, showing an EC50/1d of 3.60 mg/

dx.doi.org/10.1021/jf302075w | J. Agric. Food Chem. 2012, 60, 7345−7351

L; conversely, moving the carbonyl group from the 2 to the 3 position, we did not measured any activity.

We also observed a toxin-mediated fast nematode paralytic killing that correlates with the concentration of heteroaromatic aldehydes. Consistent with our previous report on the nematicidal activity of (*E*,*E*)-2,4-decadienal and (*E*)-2-decenal,[4] we observed the nematodes treated with heteroaromatic aldehydes were paralyzed or died in a straight shape. On the other hand, in our experiments nematodes treated with the organophosphorous fosthiazate were paralyzed in a coiling shape, as previously reported.[4] Wiratno et al. evidenced that when *M. incognita* nematodes were treated with clove methanol ethanol extract, they died in straight or bent shapes similar to those killed by the pyrethroid deltamethrin.[25] Moreover, Kong et al. evidenced that pinewood nematode treated with muscle activity blockers levamisole or morantal tartrate were paralyzed in semicircular and coiling shapes, respectively.[26] These facts suggest a different mode of action of heteroaromatic aldehydes.

In particular, from a structural point of view, the most active compounds, thiophene-2,5-dicarbaldehyde and 2-pyridinecarbaldehyde (Figure 2), share some interesting features. In fact, they possess high aromatic scaffolds and both support two hydrogen-bond acceptors that seem to be placed in a suitable position. Many reasons might be adduced for the lack of activity of the other compounds showed in Figure 2. In particular, different bond geometry, weaker hydrogen-bond acceptor strength, and different oxidation state together with a reduced electrophilicity of the carbon atom might be involved.

Besides, heteroaromatic aldehydes are relatively reactive compounds due to the presence of the carbonyl carbon atom, an electrophilic site that may easily react with primary amino and thiol groups, resulting in formation of substituted imines, called Schiff bases and hemithioacetals, respectively. According to this chemical behavior, the cuticle damage that occurs when nematodes are treated with furfural at 100 mg/L might be adducted to a nucleophilic addition of the cuticle amino or thiol residues the carbonyl functionality of the aldehyde. As previously reported, this interaction leads to evident cuticle and internal damage followed by leakage of the internal fluid nematode material.[3] Similar nematode cuticle damage was reported for *Panagrellus redivivus* caused by a unique fungal structure on the vegetative hyphae of *Coprinus comatus*.[27,28] Additionally, Niu et al. reported that aurovertin-type metabolites from the fungal strain *Pochonia chlamydosporia* lead to destruction of the internal structure of the nematode *P. redivivus*.[29] Aurovertin-type metabolites produced by the strain of *C. arbuscolo* are regarded as potent inhibitors of ATP-synthesis and ATP-hydrolysis uncoupling oxidative phosporylation.[30,31] Moreover, Mahajan-Miklos[32] reported production from *Pseudomonas aeruginosa* of small diffusible secondary nematicidal phenazine−pyocyanin metabolites that act as a potent inhibitors of human V-ATPase.[33] Even if the mode of action of heteroaromatic aldehydes tested in our study is not still elucidated, we suppose that, due to their structural relationship, they work similarly to auvertin B, an inhibitor of the vacuolar ATPase enzymes. Vacuolar-type ATPase proton pumps play an essential role in nematode nutrition, osmoregulation, cuticle synthesis, neurobiology, and reproduction[34] and for these reasons can be an effective pesticide-target against parasitic nematodes.

AUTHOR INFORMATION

Corresponding Author

*Phone: +390706758617. Fax: +390706758612. E-mail: caboni@unica.it.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to Prof. Marco Ciufolini from the University of British Columbia for a number of valuable suggestions and discussions on this study.

ABBREVIATIONS USED

LME, leaves methanol extract; SME, stems methanol extract; BME, buds methanol extract

REFERENCES

(1) Seigler, D. *Plant Secondary Metabolism*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1998.

(2) Tuomi, J. Toward integration of plant defense theories. *Trends Ecol. Evol.* **1992**, *7*, 365−367.

(3) Soler-Serratosa, N.; Kokalis-Burelle, N.; Rodriguez-Kabana, R.; Weaver, C. F.; King, P. S. Allelochemicals for control of plant-parasitic nematodes. 1. *In vivo* nematicidal efficacy of thymol and thymol/benzaldeyde combinations. *Nematropica* **1996**, *26*, 57−71.

(4) Caboni, P.; Ntalli, N. G.; Aissani, N.; Cavoski, I.; Angioni, A. Nematicidal activity of (E,E)-2,4-decadienal and (E)-2-decenal from *Ailanthus altissima* against *Meloidogyne javanica*. *J. Agric. Food Chem.* **2012**, *60* (4), 1146−51.

(5) Ntalli, N. G.; Manconi, F.; Leonti, M.; Maxia, A.; Caboni, P. Aliphatic ketones from *Ruta chalepensis* (Rutaceae) induce paralysis on root knot nematodes. *J. Agric. Food Chem.* **2011**, *13* (59), 7098−103.

(6) 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* (21), 11390−11394.

(7) Cavoski, I.; Chami, Z.Al.; Bouzebboudja, F.; Sasanelli, N.; Simeone, V.; Mondelli, D.; Miano, T.; Sarais, G.; Ntalli, N. G.; Caboni, P. Melia azadarach controls Meloidogyne incognita and triggers plant defense mechanisms on cucumber. *Crop Prot.* **2012**, 85−90.

(8) Korayem, A. M.; Hasabo, S. A.; Ameen, H. H. Effects and mode of action of some plant extracts on certain plant parasitic nematodes. *Anz. Schaedl.-Kde. Pfl.-Schutz Umweltschutz* **1993**, *66* (2), 32−36.

(9) Sasanelli, N.; Anton, A.; Takacs, T.; D'Addabbo, T.; Birò, I.; Malov, X. Influence of arbuscular mycorrhizal fungi on the nematicidal properties of leaf extracts of *Thymus vulgaris* L. *Helminthologia* **2009**, *46* (4), 230−240.

(10) Maistrello, L.; Vaccari, G.; Sasanelli, N. Effect of chestnut tannins on the root-knot nematode *Meloidogyne javanica*. *Helminthologia* **2010**, *47* (1), 48−57.

(11) Ntalli, N. G.; Ferrari, F.; Giannakou, I.; Menkissoglu-Spiroudi, U. Synergistic and antagonistic interactions of terpenes against Meloidogyne incognita and the nematicidal activity of essential oils from seven plants indigenous to Greece. *Pest Managem. Sci.* **2011**, *67* (3), 341−351.

(12) Al-Banna, L.; Darwish, R. M.; Aburjai, T. Effect of plant extracts and essential oils on root-knot nematode. *Phytopathol. Mediterr.* **2003**, *42*, 123−128.

(13) Shahina, A. G. *Capparaceae*; CRC Press Inc.: Boca Raton, FL, 1994; p 73.

(14) Zhou, H; Jian, R; Kang, J; Huang, X; Li, Y; Zhuang, C; Yang, F; Zhang, L; Fan, X; Wu, T; Wu, X. Anti-inflammatory effects of caper (*Capparis spinosa* L.) fruit aqueous extract and the isolation of main phytochemicals. *J. Agric. Food Chem.* **2010**, *58* (24), 12717−12721.

(15) Grainge, M., Ahmed, S. *Handbook of Plants with Pest-Control Properties*; J. Wiley and Sons: New York, 1988; pp 238−248.

(16) Matthaus, B.; Ozcan, M. Glucosinolate composition of young shoots and flower buds of capers (*Capparis* species) growing wild in Turkey. *J. Agric. Food Chem.* **2002**, *50*, 7323−7325.
(17) Basile, M.; D'Addabbo, T.; Sasanelli, N.; Basile, A. C. Fumigant and systemic nematicides in nurseries. *Italus Hortus* **2003**, *10* (4), 294−296.
(18) Seck, D.; Lognay, G.; Haubruge, E.; Whatelet, J. P.; Marlier, M.; Gaspar, C.; Severin, M. Biological activity of the shrub *Boscia senegalensis* (Pers.) Lam. ex Poir. (*Capparaceae*) on stored grain insects. *J. Chem. Ecol.* **1993**, *19*, 377−389.
(19) Serra, B.; Rosa, E.; Iori, R.; Barillari, J.; Cardoso, A.; Abreu, C.; Rollin, P. *In vitro* activity of 2-phenylethyl glucosinolate, and its hydrolysis derivatives on the root-knot nematode *Globodera rostochiensis*. *Sci. Horticult.* **2002**, *92*, 75−81.
(20) Ellenby, C. Mustard oils and control of the potato-root eelworm *Heterodera rostochiensis* (Wollenweber). Further field and laboratory experiments. *Ann. Appl. Biol* **1951**, *38*, 859−875.
(21) D'Addabbo, T.; De Mastro, G.; Sasanelli, N.; Di Stefano, A.; Omidbaigi, R. Suppressive action of different cruciferous crops on the root-knot nematode *Meloidogyne incognita*. *Agroindustria* **2003**, *3*, 379−380.
(22) Sasanelli, N.; D'Addabbo, T. Effect of *Cineraria maritima*, *Ruta graveolens* and *Tagetes erecta* leaf and root extracts on Italian populations of *Meloidogyne* species. *Nematol. Mediterr* **1993**, *21*, 21−25.
(23) Puntener, W. *Manual for Field Trials in Plant Protection*, 2nd ed.; Ciba Geigy: Basel, Switzerland, 1981; pp 205.
(24) Seefeldt, S. S.; Jensen, J. E.; Fuerst, E. P.. Log-logistic analysis of herbicide dose-response relationships. *Weed Technol.* **1995**, *9*, 218−227.
(25) Wiratno, T. D.; Van den Berg, H.; Riksen, J. A. G.; Rietjens, I. M. C. M.; Djiwanti, S. R.; Kammenga, J. E.; Murk, A. J. Nematicidal Activity of Plant Extracts Against the Root-Knot Nematode, *Meloidogyne incognita*. *Open Nat. Prod. J.* **2009**, *2*, 77−85.
(26) Kong, J. O.; Lee, S. M.; Moon, Y. S.; Lee, S. G.; Ahn, Y. J. Nematicidal activity of plant essential oils against *Bursaphelenchus xylophilus* (Nematoda: *Aphelenchoididae*). *J. Asia-Pac. Entomol.* **2006**, *9*, 173−178.
(27) Luo, H.; Mo, M. H.; Huang, X. W.; Li, X.; Zhang, K. Q. *Coprinus comatus*: A basidiomycete fungus forms novel spiny structures and infects nematodes. *Mycologia* **2004**, *96*.
(28) Luo, H.; Liu, Y.; Fang, L.; Li, X.; Tang, N.; Zhang, K. *Coprinus comatus* damages nematode cuticle mechanically with spiny ball and produces potent toxins to immobilize nematodes. *Appl. Environ. Microbiol.* **2008**, 3916−3923.
(29) Niu, X. M.; Wang, Y. L.; Chu, Y. S.; Xue, H. X.; Li, N; Wei, L. X.; Mo, M. H.; Zhang, K. Q. Nematodetoxic aurovertin-type metabolites from a root-knot nematode parasitic fungus *Pochonia chlamydosporia*. *J. Agric. Food Chem.* **2010**, *58* (2), 828−34.
(30) Ebel, R.; Lardy, H. Influence of aurovertin on mitochondrial ATPase activity. *J. Biol. Chem.* **1975**, *250*, 4992−4995.
(31) Van Raaij, M. J.; Abrahams, J. P.; Leslie, A. G. W.; Walker, J. E. The structure of bovine Fl-ATPase complexed with the antibiotic inhibitor aurovertin B. *Proc. Natl. Acad. Sci. U.S.A* **1996**, *93*, 6913−6917.
(32) Mahajan-Miklos, S.; Tan, M. W.; Rahme, L. G.; Ausubel, F. M. Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa-Caenorhabditis elegans* phatogenesis model. *Cell* **1999**, *96*, 47−56.
(33) Ran, H.; Hassett, D. J.; Lau, G. W. Human targets of *Pseudomonas aeruginosa* pyocyanin. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (24), 14315−14320.
(34) Knight, A. J.; Behm, C. A. Minireview: The role of the vacuolar ATPase in nematodes. *Exp. Parasitol.* **2011**, in press.