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Nematicidal Activity of Allylisothiocyanate from Horseradish (*Armoracia rusticana*) Roots against *Meloidogyne incognita*

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ABSTRACT: In recent years, there has been a great development in the search for new natural pesticides for crop protection aiming a partial or total replacement of currently used chemical nematicides. Glucosinolate breakdown products are volatile and are therefore good candidates for nematodes fumigants. In this article, the methanol-aqueous extract (1:1, w/v) of horseradish (*Armoracia rusticana*) fresh roots (MAH) was in vitro tested for nematicidal activity against second stage (J2) *Meloidogyne incognita*. The EC_{50} of MAH after 3 days of J2 immersion in test solutions was 251 ± 46 mg/L. The chemical composition analysis of the extract carried out by the GC-MS technique showed that allylisothicyanate was the most abundant compound. This pure compound induced J2 paralysis with an EC_{50} of 52.6 ± 45.6 and 6.6 ± 3.4 mg/L after 1 h and 3 days of incubation. The use of LC-MS/MS showed for the first time that horseradish root is rich in polyphenols. The study of isothiocyanate degradation in soil showed that allylisothiocyanate was the most quickly degradable compound (half-life <10 min), whereas no significant differences in half-life time were noted between degradation in regular and autoclaved soil.

KEYWORDS: *root-knot nematodes, GC-MS, LC-MS/MS, natural nematicides, Armoracia rusticana, allylisothiocyanate, isothiocyanates degradation*

INTRODUCTION

Plants may produce compounds that directly or indirectly affect their biological environment. These compounds are called allelochemicals and have a role in the growth, health, and behavior of other organisms.[1] One reason for interest in allelochemicals is their potential for use in alternative pest management systems. Many scientific studies have reported data on the biological activity of plant secondary metabolites against root-knot nematodes.[2] We recently discovered that (*E*,*E*)-2-4-decadienal and (*E*)-2-decenal from *Ailanthus altissima* are active against *Meloidogyne javanica.* Moreover, 2-thiophenecarboxaldehyde and methyl isothiocyanate from *Capparis spinosa* are active against *Meloidogyne incognita*.[3,4]

Horseradish (*Armoracia rusticana*) is a perennial crop, probably native to southeastern Europe and western Asia, belonging to the genus *Armoracia* of the *Brassicaceae* family. Its roots are popularly used as a pungent spice and as an indispensable material for producing horseradish paste and pungent sauces, and it is a common substitute of wasabi in Japanese traditional sushi.[5] It is also used to treat nonspecific urinary tract infections and seems to inhibit the growth of colon and lung cancer cells.[6,7] This plant contains several isothiocyanates, which are known for their antibacterial, antifungal, and insecticidal activities.[8]

Glucosinolates are a class of secondary compounds widely distributed throughout the *Cruciferae*. Glucosinolates are D-thioglucosides distinguished from one another by the differences in their organic side chains (R groups), according to which glucosinolates are grouped as either aliphatic, aromatic, or indole forms. They occur in all plant tissues and degrade via enzymatic hydrolysis. Tissue damage brings glucosinolates in contact with myrosinase (EC 3.2.1.147), and as a result, glucose and sulfates are released along with several toxic and pungent products, such as isothiocyanates, nitriles, and oxazolidinethiones, depending on the parent glucosinolate, pH, and other factors.[9,10] Isothiocyanates are general biocides whose activity is based on irreversible interactions with proteins.[11]

Much of scientific research on horseradish extracts deals with the nematicidal activities of different parts of this plant, but to our knowledge (after performing a literary survey), there is no report available on the nematicidal activity of *Armoracia rusticana* root extracts and its constituents against *M. incognita.*

In the present investigation, we report for the first time (1) the GC-MS and LC-MS/MS chemical characterization of the root of *A. rusticana* methanol-aqueous extract (MAH), (2) the study of the in vitro biological activity (EC_{50}) of the MAH and its compounds against *M. incognita* second-stage juveniles, and (3) the degradation profile of isothiocyanates in soil.

MATERIALS AND METHODS

Chemicals. Allylisothiocyanate, benzylisothiocyanate, methylisothiocyanate, phenylisothiocyanate, and fosthiazate were obtained from Sigma-Aldrich (Milano, Italy), methanol, formic acid, and water were of high-performance liquid chromatography (HPLC)-grade.

Extraction of Plant Materials. Roots of *A. rusticana* (5 kg) were collected from Ferrara (Emilia-Romagna, Italy) in June 2012. They were sealed in paper bags, stored at room temperature, and kept in the dark until use. Voucher specimens were deposited at the Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy, for species identification. One hundred grams of fresh roots were

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Table 1. Multiple Reaction Monitoring Transitions (LC-MS/MS) and Phenolic Compounds in *Armoracia rusticana*

			first transition		second transition			
compd	MW	precursor mass (m/z)	mass (m/z)	CE (V)	mass (m/z)	CE (V)	R_t (min)	concn (μg/g)
malic acid	134	$[M - H]^-$	133.0→71.0	(13.5)	133.0→114.8	(9.0)	8.47	307 ± 245
gallic acid	170	$[M - H]^-$	169.0→124.8	(12.5)			18.04	1.89 ± 0.62
ferulic acid	194	$[M - H]^-$	193.0→177.7	(16.0)	193.0→133.8	(18.0)	18.88	35.1 ± 20.3
(−)epigallocatechin-3-gallate	458	$[M - H]^-$	457→168.7	(15.5)	457→304.9	(17.5)	20.56	7.82 ± 5.43
myricitrin	464	$[M - H]^-$	463→286.9	(42)	463→317.0	(21.0)	22.85	5.09 ± 1.95

ground and extracted with a 100 mL mixture of methanol and water (1:1, w/v) for 2 days. Ten milliliters of the extract was filtered through a Whatman no. 40 filter paper and centrifuged for 15 min at 13000 rpm. The extract was then sterilized by filtration using a 0.45 μm filter and then analyzed by GC-MS and LC-MS/MS. Plant material was also dried at 105 °C for 24 h, and the moisture was found to be 69.21%.

GC-MS Analysis. *Roots Extract.* Chromatographic separation and identification of components of methanol aqueous extracts of *A. rusticana* were performed on a gas chromatograph Varian model 3800 equipped with a Varian 7800 autosampler, a split/splitless injector Varian 1079, and an ion trap mass detector (ITMS) 2000. The analytical column was a Varian VF5m s (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Varian, Milan, Italy). Helium was the carrier gas at 1 mL/min. A 1 μL sample, previously dehydrated with sodium sulfate, was injected in splitless mode with a purge valve on at 1 min. The injector temperature was set at 200 °C. The mass spectrometer was calibrated weekly, following the autotune test of the software (Saturn GC-MS Workstation 5.41). The mass spectrometer detector was operated in electron ionization positive mode. Trap, manifold, and transfer line temperatures were at 200, 80, and 200 °C, respectively. The oven was programmed as follows: 50 °C (1 min), raised to 100 °C (5 °C/min), and held for 1 min, then raised to 180 °C (20 °C/min), and held for 4 min. Qualitative analysis was performed in the scan mode (50−550 amu). Peak identification was made comparing full mass spectra and retention times from authentic standards and the NIST MS Spectra Library (The NIST Mass Spectral Search Program for the NIST\EPA\NIH mass Spectral Library, version 2.0, build 12/2000).

Isothiocyanates Degradation Studies in Soils. A soil sample (20 kg) was collected from an open field in Uta, Sardinia (Italy) in 2012 from the top 20 cm. Macroresidual materials, macro faunal remains, and stones were accurately removed, and larger soil aggregates were manually fragmented into smaller ones, prior to the subsequent fractionation procedure. Soils were air-dried and sieved <2 mm, separated into pots of 200 g, divided into two categories, autoclaved (11 min at 121 °C, 15 psi) to destroy microbes (such as bacteria, actinomycetes, fungi, and protozoa), and nonautoclaved, and then spiked with 100 μL of 100 g/L of isothiocyanates mixture and kept in the dark at room temperature. At 0, 10, 20, 30, 60, 120, and 240 min of incubation, 200 g of soil was extracted with 200 mL of methanol, sonicated for 15 min, filtered through a Whatman no. 40 filter paper, and centrifuged for 15 min at 13000 rpm and then injected for GC-MS analysis. The analysis was performed as in the selection ion storage (SIS) mode using the following ions: m/z 99 for allylisothiocyanate, m/z 135 for phenylisothiocyanate, and m/z 149 for benzylisothiocyanate, integrating the peak area of the GC-MS chromatograms versus concentration. Four replicates were analyzed.

LC-MS/MS Analysis. A Varian 1200 L triple-quadrupole tandem mass spectrometer (Palo Alto, CA) coupled with a ProStar 410 autosampler and two ProStar 210 pumps and a 1200 L triple-quadrupole mass spectrometer was used with an electrospray ionization (ESI) source. The Varian MS workstation, version 6.7, software was used for data acquisition and processing. Chromatographic separation was performed on Zorbax Column Synergi 4 μm MAX-RP 80A (150 × 4.6 mm) (Phenomenex). The mobile phase consisted of (A) double distilled water and (B) methanol containing 0.1% formic acid. The solvent gradient started at 10% of B reaching 100% in 20 min and 100% in 25 min followed by post-time isocratic conditions for 5 min at 10% of B before the next injection. The mobile phase, previously degassed with high-purity helium, was pumped at a flow rate of 0.3 mL/min, and the injection volume was 10 μL. ESI was operated in negative ion mode. The electrospray capillary potential was set to −40 V, the needle at −4500 V, and the shield at −600 V. Nitrogen at 48 mTorr and 41 °C was used as a drying gas for solvent evaporation. The atmospheric pressure ionization (API) housing was kept at 50 °C. Parent compounds were subjected to collision-induced dissociation using argon at 2.40 mTorr in multiple reaction monitoring (MRM) in the negative mode. Table 1 reports the observed mass transitions and collision energy used for quantitation of different phenolics. The scan time was 1 s, and the detector multiplier voltage was set to 2000 V, with an isolation width of m/z 1 for quadrupole 1 and m/z 1.9 for quadrupole 3.

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.[12] Batches were then incubated in a growth cabinet at 25 ± 2 °C in dark conditions. Emerging juveniles J2 (24 h) were removed and collected every day and used for the experiments. MAH was tested against root-knot nematodes at a dose range of 10−5000 mg/L and assessed for toxicity at 72 h using fosthiazate as a chemical control.

Allylisothiocyanate, found in MAH, and other aliphatic and aromatic isothiocyanates such as benzylisothiocyanate, methylisothiocyanate, and phenylisothiocyanate (Figure 1) were tested against J2 at

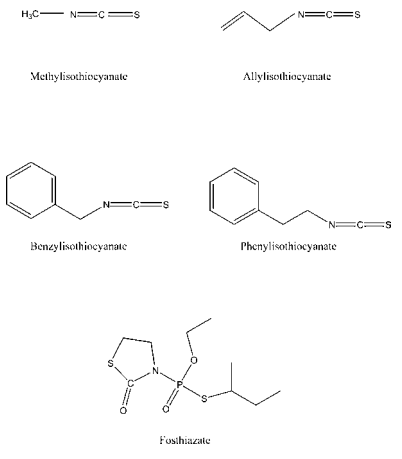

Figure 1. Chemical structures of tested compounds.

a dose range of 1.0−1000 mg/L using the organophosphorus fosthiazate as a chemical control. In the same experiment, malic acid, gallic acid, ferulic acid, and (−)epigallocatechin-3-gallate, found by LC-MS/MS in the MAH extract, were tested against J2.

Stock solutions of pure compounds were prepared in methanol to overcome insolubility, whereas Tween 20 in twice distilled water was used for further dilution. The final concentration of methanol in each well never exceeded 1% (v/v) since preliminary experiments showed that this concentration was not toxic to nematodes. Distilled water as well as a mixture of methanol and Tween (0.3% v/v) served as

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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 a 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 methanol−water extracts of *A. rusticana* roots. J2 were ranked in two distinct categories: motile or paralyzed/dead. Paralysis experiments were performed four times, and every treatment was replicated six times.

Statistical Analysis. Since paralysis in solvent (methanol, Tween-20) did not differ significantly from that observed in distilled water, the percentages of paralyzed/dead 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:[13] 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:[14]

$$Y = C + (D - C)/\{1 + \exp[b(\log(x) - \log(EC_{50}))]\}$$

where C = the lower limit, D = the upper limit, b = the slope at 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 period was used to calculate the EC_{50} value (4 replicates for degradation in soil). Mean data values were reported with the respective standard deviations.

RESULTS AND DISCUSSION

When MAH was tested against *M. incognita*, a clear dose−response relationship was established and significant paralysis/death of J2 was evident after 3 days of exposure with a calculated $EC_{50/3d}$ value of 250.7 ± 45.8 mg/L. By GC-MS analysis, we were able to quantify the most abundant plant metabolite, allylisothiocyanate, at a concentration of 10893 ± 7810 μg/g (30.1%), while the identification, by the NIST library of other constituents of the extract, was unsuccessful. Allylisothiocyanate applied against J2 was active with an $EC50/_{1d}$ of 6.6 ± 3.4 mg/L. This is the first report of the nematicidal activity of allylisothiocyanate as a constituent of *A. rusticana* against *M. incognita*. Zasada et al. tested this compound against *M. javanica* and found an LC_{50} of 10 mg/L.[15] Allylisothiocyanate was also found in mustard and is a practical alternative to fenamiphos, one of the most widely used nematicides in the turfgrass industry.[16]

We then tested other isothiocyanates with structure similarities to the allylisothiocyanate, and the respective EC_{50} values are presented in Table 2. Slight structural differences can confer important different nematicidal effects, confirming that biological activity is a function not only of the concentration of the product but also of the chemical properties of the alkyl side chain.[17] When the double bound of the alkyl side chain was substituted by a benzene moiety as in the case of benzenisothiocyanate, the compound was three times more active. We also observed that J2 treated with different isothiocyanates were paralyzed or died in a straight shape. However, in our experiments nematodes treated with the organophosphorous fosthiazate were paralyzed in a coiling shape, as previously reported by Kong et al. when they treated the pinewood nematode with muscle activity blockers levamisole and morantal tartrate.[18]

Table 2. EC_{50} Values (mg/L) (± SD, n = 4) of Pure Compounds against *M. incognita* at 1 h and 1 day after Treatment

compd	1 h, EC_{50} (mg/L)	1 d, EC_{50} (mg/L)
allylisothiocyanate[a]	52.6 ± 45.6	6.6 ± 3.4
benzylisothiocyanate[a]	11.7 ± 7.2	1.9 ± 1.1
methylisothiocyanate	54.4 ± 16.2	7.9 ± 1.6
phenylisothiocyanate	>1000	>1000
malic acid	>1000	>1000
gallic acid	>1000	>1000
ferulic acid	>1000	>1000
myricitrin	NT	NT
(−)epigallocatechin-3-gallate	>1000	>1000
fosthiazate	3.3 ± 1.0	0.40 ± 0.3

[a]These compounds showed 100% mortality after 24 h of juvenile immersion in 100 mg/L of solution. NT = not tested.

As electrophiles, isothiocyanates and heteroaromatic aldehydes are capable of reacting with sulfhydryl groups and binding to protein amino and thiols groups by interaction with cystein, and these modifications may alter protein functions.[19,20] A recent study showed that isothiocyanates can disrupt tubulin polymerization and spindle assembly, which in turn causes mitotic arrest.[21] In addition, heteromatic aldehydes such as salicylaldehyde at the concentration of 1 mM were found to form an adduct with the synthetic peptide NAacetylAEEQCTSCVQLQCP of the nematode cuticle leading to macroscopic fractures of the nematode body wall.[22]

Malic acid, gallic acid, ferulic acid, and (−)epigallocatechin-3-gallate, determined by LC-MS/MS in the MAH extract, were not active at the doses 100−1000 mg/L (Table 2) as previously reported by Aoudia et al.[23]

Because of the high volatility of isothiocyanates, we then tested the persistence of structurally related isothiocyanates in soils, and no significant differences on half-life time in autoclaved and nonautoclaved soil by GC-MS analysis were detected; however, high volatility is noted (Figure 2). This fact shows that isothiocyanate degradation in soil is not due to biological factors. In all cases, allylisothiocyanate was the most volatile compound with a half-life time lower than 10 min followed by phenylisothiocyanate and benzylisothiocyanate. Our results are similar to those by Borek et al. who saw no effect on the transformation of allylisothiocyanate in autoclaved soil.[24] The degradation of chemicals depends mostly on soil composition, moisture, and temperature. Physical and chemical properties of the soil used in this study are shown in Table 3. Faster rates of allylisothiocyanate disappearance occurred in soils with high organic carbon contents and total nitrogen concentrations, reduced soil moisture, and high temperature.[24] In both sterile and nonsterile soils occurred an initial fast decrease rate followed by a much slower decline, which clearly indicates the rather complex chemical process of isothiocyanate degradation in soil. This initial rate was most likely a combination of volatilization, irreversible partitioning into the organic matter of the soil, and reactivity with nucleophilic groups.[25] This was supported by the observation that it was possible, at the first sampling time, to recover more benzylisothiocyanate than phenylisothiocyanate and allylisothiocyanate taking into account that benzylisothiocyanate is not

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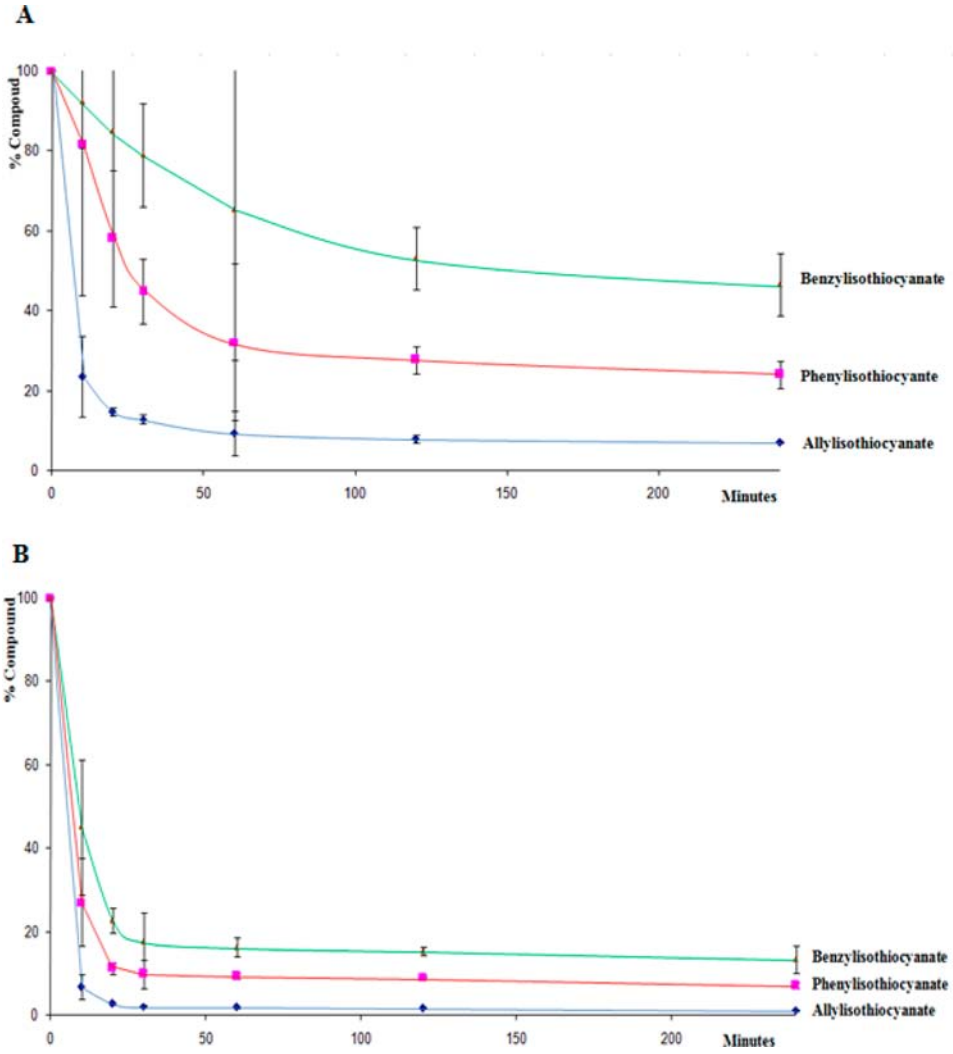

Figure 2. Isothiocyanate degradation profile in soil: (A) nonautoclaved soil and (B) autoclaved soil.

Table 3. Physicochemical Properties of Soil

parameter	g/kg
sand	442
silt	196
clay	362
total organic substance	26.2
total nitrogen	1.56
pH in H_2O	8.25

very reactive. Autoclaving the soil did not affect the half-life probably due to the inhibiting effect of isothiocyanates on bacterial growth, just as reported by Klose et al. 2006,[26] or also due to the fast volatility of isothiocyanates not permitting degradation by bacteria.

This article deals with the study of the nematicidal activity of the horseradish root methanol−water extract against *M. incognita*. Horseradish roots are rich in allylisothiocyanate, which has nematicidal activity against this root-knot nematode and allows us to use this plant for crop protection against pests. Isothiocyanates are high volatile compounds that are quickly degraded in soil, a fact that is desirable from an environmental point of view, but if they are to be utilized for pest control, fast degradation may imply that they are not present long enough to have the desired effect on pests. Further studies on the formulation of isothiocyanates shall be done to improve their stability in the soil. As a perspective, we will study the mode of action of isothiocyanates in silico using protein modeling.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Whittaker, R. H.; Feeny, P. P. Allelochemics: chemical interactions between species. *Science* **1971**, *171*, 757−770.

(2) Ntalli, N. G.; Caboni, P. Botanical nematicides: a review. *J. Agric. Food Chem.* **2012**, *60*, 9929−9940.

(3) 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), 1146−1151.

(4) Caboni, P.; Sarais, G.; Aissani, N.; Tocco, G.; Sasanelli, N.; Liori, B.; Carta, A.; Angioni, A. Nematicidal activity of 2-thiophenecarboxaldehyde and methylisothiocyanate from Caper (*Capparis spinosa*) against *Melodogyne incognita*. *J. Agric. Food Chem.* **2012**, *60* (30), 7345−7351.
(5) Hudocecova, A.; Hasplova, K.; Kellowska, L.; Ikreniova, I.; Miadokova, E.; Galova, E.; Horvathova, E.; Vaculcikova, D. *Gentiana asclepiadea* and *Armoracia rusticana* can modulate the adaptive response induced by zeocin in human lymphocytes. *Neoplasma* **2012**, *59* (1), 62−69.
(6) Agneta, R.; Rivelli, A. R.; Ventrella, E.; Lelario, F.; Sarli, G.; Bufo, S. A. Investigation of glucosinolate profile and quantitative aspects in sprouts and roots of horseradish (*Armoracia rusticana*) using LC-ESI-Hybrid linear ion trap with Fourier transform ion cyclotron resonance mass spectrometry and infrared multiphoton dissociation. *J. Agric. Food Chem.* **2012**, *60*, 7474−7478.
(7) Weil, M. J.; Zhang, Y.; Nair, M. G. Tumor cell proliferation and cyclooxygenase inhibitory constituents in horseradish (*Armoracia rusticana*) and wasabi (*Wasabia japonica*). *J. Agric. Food Chem.* **2005**, *53*, 1440−1444.
(8) Tedeschi, P.; Leis, M.; Pezzi, M.; Civolani, S.; Maietti, A.; Brandolini, V. Insecticidal activity and fungitoxicity of plant extracts and components of horseradish (*Armoracia rusticana*) and garlic (*Allium sativum*). *J. Environ. Sci. Health, Part B* **2011**, *46*, 486−490.
(9) Bones, A. M.; Rossiter, J. T. The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry* **2006**, *67*, 1053−1067.
(10) Wittstock, U.; Halkier, B. A. Glucosinolate research in the *Arabidopsis* era. *Trends Plant Sci.* **2002**, *7*, 263−270.
(11) Brown, P. D.; Morra, M. J. Control of soil-borne plant pests using glucosinolate-containing plants. *Adv. Agron.* **1997**, *61*, 167−231.
(12) Sasanelli, N.; D'Addabbo, T. Effect of *Cineraria maritime*, *Ruta graveolens* and *Tagetes erecta* leaf and root extracts on Italian populations of Meloidogyne species. *Nematol. Mediterr.* **1993**, *21*, 21−25.
(13) Puntener, W. (1981) *Manual for Field Trials in Plant Protection*, 2nd ed.; Ciba Geigy, Basel, Switzerland, p 205.
(14) Seefeldt, S. S.; Jensen, J. E.; Fuerst, E. P. Log-logistic analysis of herbicide rate response relationships. *Weed Technol.* **1995**, *9*, 218−227.
(15) Zasada, I. A.; Ferris, H. Sensitivity of *Meloidogyne javanica* and *Tylenchulus semipenetrans* to isothiocyanates in laboratory assays. *Nematology* **2003**, *93*, 747−750.
(16) Fleming, C. C.; Turner, S. J.; Hunt, M. Management of root knot nematodes in turfgrass using mustard formulations and biostimulants. *Commun. Agric. Appl. Biol. Sci.* **2006**, *71*, 653−658.
(17) Lazzeri, L.; Tacconi, R.; Palmieri, S. In vitro activity of some glucosinolates and their reaction products toward a population of the nematode *Heterodera schachtii*. *J. Agric. Food Chem.* **1993**, *41*, 825−829.
(18) 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.
(19) Nakamura, Y.; Miyoshi, M. Electrophiles in food; the current status of isothiocyanates and their chemical biology. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 242−255.
(20) Mi, L.; Xiao, Z.; Hood, B. L.; Dakshanamurthy, S.; Wang, X.; Govind, S.; Conrads, T. P.; Veenstra, T. D.; Chung, F. L. Covalent binding to tubulin by isothiocyanates: a mechanism of cell growth arrest and apoptosis. *J. Biol. Chem.* **2008**, *283*, 22136−22146.
(21) Jackson, S. J.; Singletary, K. W. Sulforaphane: a naturally occurring mammary carcinoma mitotic inhibitor, which disrupts tubulin polymerization. *Carcinogenesis* **2004**, *25*, 219−227.
(22) Caboni, P.; Aissani, N.; Cabras, T.; Falqui, A.; Marotta, R.; Liori, B.; Ntalli, N.; Sarais, G.; Sasanelli, N.; Tocco, G. Potent nematicidal activity of phthalaldehyde, salicylaldehyde, and cinnamic aldehyde against *Meloidogyne incognita*. *J. Agric. Food Chem.* **2013**, in press.
(23) Aoudia, H.; Ntalli, N.; Aissani, N.; Yahiaoui-Zaidi, R.; Caboni, P. Nematotoxic phenolic compounds from *Melia azedarach* against *Meloidogyne incognita*. *J. Agric. Food Chem.* **2012**, *28* (60), 11675−80.
(24) Borek, V.; Morra, M. J.; Brown, P. D.; McCaffrey, J. P Transformation of the glucosinolate-derived allelochemicals allyl isothiocyanate and allylnitrile in soil. *J. Agric. Food Chem.* **1995**, *43*, 1935−1940.
(25) Gimsing, A. L.; Strobel, B. W.; Hansen, H. C. B. Degradation and sorption of 2-propenyl and benzy lisothiocyanate in soil. *Environ. Toxicol. Chem.* **2009**, *6*, 1178−1184.
(26) Klose, S.; Acosta-Martinez, V.; Ajwa, H. A. Microbial community composition and enzyme activities in a sandy loam soil after fumigation with methyl bromide or alternative biocides. *Soil Biol. Biochem.* **2006**, *38*, 1243−1254.