exeley
Article | DOI: 10.21307/jofnem-2018-017

JOURNAL OF NEMATOLOGY
Issue 3 | Vol. 50 (2018)

Nematicidal Weeds, *Solanum nigrum* and *Datura stramonium*

Chrisostomos Oplos,[1] Kodjo Eloh,[2] Urania-Menkissoglu Spiroudi,[1] Caboni Pierluigi,[2] and Nikoletta Ntalli[3]*

[1]Pesticide Science Laboratory, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.

[2]Department of Life and Environmental Sciences, University of Cagliari, 09124 Cagliari, Italy.

[3]Laboratory of Biological Control of Pesticides, Department of Pesticide Control & Phytopharmacy, Benaki Phytopathological Institute, Athens 14561, Greece.

*Email: nntali@agro.auth.gr.

This article was edited by James A. LaMondia.

Received for publication December 27, 2017.

Abstract

We investigated *Solanum nigrum* (seeds) and *Datura stramonium* (shoots) against root-knot nematodes in terms of J2 paralysis and egg hatch inhibition (methanol extract), as well as inhibition of nematode development in host roots (soil amending with either *S. nigrum* seeds' or *D. stramonium* shoots' meal). *Datura stramonium* was found equally effective at inhibiting motility of *Meloidogyne incognita* and *Meloidogyne javanica* (both EC_{50} = 427 µg mL^{-1} at 3 day), but inhibition occurred more quickly for *M. incognita* (1 day). *Solanum nigrum* was faster and more effective at inhibiting motility of *M. incognita* than *M. javanica* (EC_{50} = 481 and 954 µg mL^{-1} at 3 day, respectively). *Datura stramonium* was slower, but eventually more potent in decreasing egg hatch and cell division in *M. incognita* eggs, than *S. nigrum*. Specifically, *D. stramonium* significantly inhibited cell division in eggs immersed in at least 100 and 1 µg mL^{-1} at Day 6 and 10, respectively. *Solanum nigrum* impeded cell division in un-differentiated eggs immersed in not less than 10 and 100 µg mL^{-1} after days 2 and 6, respectively. Both extracts were similar in suppressing J2 exclosure but *D. stramonium* was effective in smaller test concentrations. Specifically, *D. stramonium* suppressed J2 emerging from eggs immersed in 10 µg mL^{-1} at day2, and in at least 1 µg mL^{-1} at day 6. *Solanum nigrum* significantly reduced J2 hatch from eggs immersed in a minimum of 100 µg mL^{-1} at day 2 and not less than 1,000 µg mL^{-1} at day 6. In pots, powdered *S. nigrum* seeds meal was more active than *D. stramonium* and the respective EC_{50} females/*g* values for *M. incognita* were 1.13 and 11.4 mg g^{-1} of soil, respectively. The chemical composition of active extracts was determined after derivatization by GC–MS. Chemical analysis of active extracts showed the presence of fatty acids with known nematicidal activity.

Key words

Black nightshade, Jimsonweed, Root-knot nematodes, Weeds.

Weeds compete with crop plants and soil organisms for resourses through the production of allelochemicals like phenolic acids, terpenes, terpenoids, glycosides, alkaloids, and flavonoids (Whittaker and Feeny, 1971; Blum, 1996; Keating, 1999). A major phyto-nematode control research issue is the study of herbal preparations rich in allelochemicals with nematicidal activity, of no adverse effects to non-target organisms and easy biodegradability. The use of green manures as soil bioamendments may be a suitable nematode control tool for many crop systems, especially if the botanical species to be incorporated are readily available *in situ*, like weeds. *Solanum nigrum* Linn. and *Datura stramonium* Linn., commonly known as black nightshade and jimsonweed, are two Solanaceous, highly invasive and globally distributed weeds that exhibit a range of biological properties (Zhou et al., 2012; Abbasi et al., 2015; Sher et al., 2015).

Although *S. nigrum* can be infected by *Meloidogyne incognita* (Robab et al., 2012) it also exhibits

nematicidal activity. Specifically, its dried ground seed powder incorporated in soil at the rate of 5 g kg^{-1} lessens root galling and increases host shoot length (Radwan et al., 2012). Moreover, the water extract of *S. nigrum* at a concentration of 10 mg ml^{-1} induces morphological changes in the body structure of the root-lesion nematode *Pratylenchus goodeyi*, greatly affects movement and causes mortality (Gouveia et al., 2014). Interestingly, root extracts of *S. nigrum* are traditionally used in the treatment of animal worms and abdominal pain (Jagtap et al., 2013).

Solanum nigrum is a major source of various chemical groups of nematicidal compounds like alkaloids (Jagtap et al., 2013; Sammani et al., 2013), glycoalkaloids (Li et al., 2007; Ding et al., 2013), saponins (Jagtap et al., 2013), phenols (Gharbi et al., 2017), fatty acids (Dhellot et al., 2006; Mohy-ud-din et al., 2010) and tannins (Jagtap et al., 2013). An alkaloid named drupacine was found to exhibit an EC_{50} value of 76.3 µg ml^{-1} on *M. incognita* second stage juveniles (J2) and to reduce egg hatch by 36% after immersion in 1.0 mg ml^{-1} (Wen et al., 2013). Similarly, 4-quinolone waltherione and waltherione A, have been reported to have larvicidal activity against *M. incognita* (EC_{50} values of 0.09 and 0.27 µg ml^{-1} at 48 h) and egg hatch inhibition activity (91.9 and 87.4% after 7 days of exposure to 1.25 µg ml^{-1}) (Jang et al., 2015). Saponins like solanigroside A and solanigroside B (Zhou et al., 2007) as well as oleanane-type triterpenoid saponins exhibit LC_{50} values against *M. incognita* ranging from 70.1 to 94.7 µg ml^{-1} after 48 h (Li et al., 2013); while a saponin based commercial nematicide from *Quillaja saponaria* has been registered for nematode control in Europe (Giannakou, 2011). 4-methylphenol is of significant *in vitro* activity against *M. javanica* (Yang et al., 2015) while Zhang and co-workers have demonstrated that fatty acids like caproic, caprylic, capric, lauric, myristic, and palmitic cause significantly high mortality to *M. incognita* J2 (Zhang et al., 2012). Tannic acid has been proven nematicidal as well (Hewlett et al., 1997). We also, in our previous studies, have demonstrated that acetic and hexanoic acid, as components of *Melia azedarach*, are effective against root-knot nematodes in terms of J2 paralysis activity (Ntalli et al., 2010).

Similar to *S. nigrum, D. stramonium* is a host for root-knot nematodes and even increases populations of *Meloidogyne* species if not controlled effectively (Ntidi et al., 2012). Nonetheless, hot water and ethanol extracts of *D. stramonium* seeds tested at 25 to 100 mg ml^{-1} caused 75% to 100% mortality of *M. incognita* J2 (Chaudhary et al., 2013). Similarly, leaf and stem extracts of *D. stramonium* tested at 500 mg L^{-1} against J2 resulted in relatively high mortality rates of 68 and 70% after 72 h of exposure (Elbadri et al., 2008). When tested in pot experiments, dried ground leaves of *D. stramonium* mixed with soil at the rate of 1 to 10 g kg-^{1} soil significantly suppressed *M. incognita* populations and root galling as they decomposed, but high rates proved to be phytotoxic (Radwan et al., 2006). Pre-plant treatments with *D. stramonium* leaf extracts at 0.5% to 1% significantly reduced gall numbers (Mateeva and Ivanova, 2000). Furthermore, aqueous leaf extracts of *D. stramonium* inhibited egg hatch and killed *M. incognita* larvae (Rao et al., 1986).

Chemical composition studies on *D. stramonium* seeds revealed N-*trans*-feruloyl tryptamine, hyoscyamilactol, scopoletin, umckalin, daturaolone, daturadiol, N-*trans*-ferulicacyl-tyramine, cleomiscosin A, fraxetin, scopolamine, 1-acetyl-7-hydrox-beta-carbol-ine, 7-hydroxy-beta-carboline1-propionic acid (Li et al., 2012). Scopolamine is a muscarinic antagonist (Lee et al., 2000) and one of the most important alkaloids present in *D. stramonium* (Ma et al., 2015).

The scope of this study was to (i) evaluate the nematicidal activity of *S. nigrum* and *D. stramonium* in terms of (a) J2 paralysis, (b) egg hatch inhibition, and (c) inhibition of nematode development in host roots and (ii) to delineate the chemical composition of active extracts after derivatization by GC–MS.

Materials and methods

Nematode rearing and collection

Populations of *M. incognita* and *M. javanica* both of Greek origin were reared on tomato (*Solanum lycopersicum* Mill.) cv. Belladonna. Freshly hatched (24 h) J2 as well as eggs of different growth stages were extracted from egg masses according to Hussey and Barker (1973) from 60 day-old (d) infested roots, to be used for the bioassays. The egg masses were hand-picked from the tomato roots under a stereoscope.

Chemicals

Methanol, chloroform, and hexane were of high-performance liquid chromatography grade. All chemical standards were obtained from Sigma-Aldrich (Milano, Italy).

Plant material extraction procedure

Dry plant material, 5 g of *S. nigrum* (seeds) and *D. stramonium* (shoots), were extracted in 50 ml methanol for 30 min in a sonicator apparatus. After exhaustive evaporation of the solvent the yields

in dry material were measured at 12.3 ± 0.07 and 12.6 ± 0.01% (w/w) for *D. stramonium* and *S. nigrum*, respectively. The extracts were used directly for bioassays with nematodes and chemical composition analysis without evaporation.

J2 paralysis bioassays

J2s were extracted as described previously after hatch in modified Baermann funnels. Hatched juveniles were discarded after the first 2 days. Thereafter, hatched J2 less than or equal to 2 days-old were used for the paralysis experiments. The *D. stramonium* and *S. nigrum* extracts were diluted in DMSO, brought to volume with water and tested for paralysis activity in Cellstar 96-well cell culture plates (Greiner Bio-One) at a ratio of 1:1 (*v/v*) with nematodes' suspension. The final concentration of DMSO in test wells did not exceed 1% (*v/v*). Distilled water served as a control together with the carrier control (DMSO). Each well contained 15 J2s and the test concentrations of both extracts ranged from 100 to 1,000 µg ml^{-1}. Border wells with J2s immersed in distilled water alone served as controls for fumigant activity test (Ntalli et al., 2011). Multiwell plates were covered to avoid evaporation and were maintained in the dark at 20°C. Juveniles were ranked into two distinct categories, moving and paralysed, with the aid of an inverted microscope (Euromex, The Netherlands) at ×40 after 1d, 2d, and 3d. After evaluation, J2 were washed through a 20 µm sieve, to remove the test compounds, and were immersed in water alone to determine if motility was regained. Numbers of motile and paralysed J2s were assessed by pricking the juvenile body with a needle, and they were counted. Nematodes that did not move at this point were considered dead. J2 paralysis bioassays were performed three times, and every treatment was replicated six times.

Egg hatch inhibition in free eggs treated with the test compounds

The egg hatch inhibition tests were performed in microwell assays (Ntalli et al., 2016). Briefly, nematodes were pipetted into 24-well cell culture plates (Greiner Bio-One), with 0.5 mL treatment at double the test concentration and 0.5 mL nematode inoculum (20 eggs) in sterile distilled water per well. *Datura stramonium* and *S. nigrum* methanol extracts were dissolved in DMSO and then brought to volume with water to reach desired concentrations. The final concentration of DMSO in test solutions did not exceed 1% as this concentration did not harm nematodes. The bioassay treatments were: 0.0 µg ml^{-1} (water control), 0.0 µg ml^{-1} (carrier control), and 1, 10, 100, and 1,000 µ g ml^{-1}, extract in the carrier. Five wells were used per treatment, and the plates were covered by plastic adhesive sheets to prevent volatiles escaping to adjacent wells. Hatch quantification was done by directly counting undifferentiated eggs and J2 in each well at day 0 using an inverted microscope at ×40. Thereafter, assessments were performed after 2, 6, 10, and 14 days. Cumulative percent J2 release was calculated using the forzmula: $((J2_{Dx} - J2_{D0})/\text{total}) \times 100$ where D_x = day after the start of the assay. Cumulative percent undifferentiated egg hatch was calculated using the formula: $((Eggs_{D0} - Eggs_{Dx})/\text{total}) \times 100$ where D_x = day after the start of the assay.

Inhibition of nematode development

Procedures were according to Ntalli et al. (2010). Briefly artificially inoculated with *M. incognita* tomato plants were then treated with powders of *S. nigrum* seeds and *D. stramonium* shoots, in a dose response from to 0.1 to 100 mg g^{-1}. After the completion of a biological cycle at 27°C, 60% RH at 16 h photoperiod, plants were uprooted and roots were stained with acid fuchsin (Byrd et al., 1983). The following variables were assessed: fresh root weight, fresh shoot weight, and total number of female nematodes and galls per gram of root at ×10 magnification under uniform illumination by transparent light. The experiment was performed twice, and the treatments were arranged in a completely randomised design with five replicates.

Sample extraction for GC–MS analysis

For small polar metabolite analysis, the following procedure was used. Powdered plant material (100 mg) was extracted with 2 mL solvent mix chloroform/methanol (2/1, *v/v*), and three replications were made. After dispersion, the whole mixture was agitated for 15 to 20 min in an orbital shaker at room temperature. The mixture was centrifuged to recover the liquid phase. The supernatant was washed with 400 µL of 0.9% KCl solution in water and vortexed for 1 min. After centrifugation at 2,000 rpm, the water phase was evaporated to dryness under a nitrogen stream. Afterwards, the residue was suspended in 50 µL of methoxyamine hydrochloride (10 mg ml^{-1}) in pyridine. After 17 h, 50 mL of *N,O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA) were added and kept for 1 hr at room temperature before 600 µL of a solution of 2-dodecanone in hexane (20 mg L^{-1}) were added and samples were GC/MS analyzed. Derivatized atropine, linoleic acid and monostearin were used for GC/MS calibration.

For the alkaloidal compounds analysis, extraction with chloroform was performed as follows. Powdered plant material (100 mg) was extracted with a mix of 5 mL of a 0.1 N sodium hydroxide in water and 5 mL of $CHCl_3$. A solution of caffeine in methanol (1 mg ml^{-1}) was added as internal standard (I.S.). After 5 min centrifugation, the chloroform phase was separated and evaporated to dryness under a nitrogen stream. The residue was suspended in 100 µL of BSTFA and kept for 1 h at 70°C for silylation. After a 10-fold dilution with hexane, samples were GC/MS analyzed.

GC–MS conditions

One microliter of derivatized plant extract was injected in splitless mode into a 6,850 gas chromatograph coupled with a mass spectrometer 5,973 Network (Agilent Technologies, Santa Clara, CA, USA) equipped with a 30 m × 0.25 mm ID silica capillary column, which was chemically bonded with 0.25 µm DB-5MS stationary phase (J&W scientific, Folsom, CA, USA). The injector temperature was kept at 200°C and the mobile phase flow was 1 mL min^{-1}. The column temperature gradient was as follows: 50°C for 10 min, then increased from 50 to 300 at a rate of 10°C min^{-1} and finally held at 300°C for 4 min. The transfer line and the ion source temperatures were respectively 280°C and 180°C. Ions were generated at 70 eV with electron ionization and were recorded at 1.6 scan sec^{-1} over the mass range *m/z* 50 to 550. GC–MS data analysis was conducted by integrating each resolved chromatogram peak and normalizing the area for the corrected total area of the chromatogram. These peaks were examined for their mass spectra and identification of the peaks was attempted using the NIST 08 library after deconvolution with AMDIS.

Statistical analysis

Treatments of motility experiments were replicated six times, and each experiment was performed twice. The percentages of paralyzed J2 observed in the microwell assays after 1 h were corrected by eliminating the natural death/paralysis in the water control according to the Schneider Orelli's formula: Corrected % = {(Mortality percent in treatment - Mortality percent in control)/(100 - Mortality percent in control)} × 100 and they were analysed (ANOVA) after being combined over time. Since the ANOVA indicated no significant treatment by time interaction, means were averaged over experiments. Corrected percentages of paralyzed J2 treated with the weed extracts were subjected to nonlinear regression analysis using the log–logistic equation proposed by Seefeldt *et al.*: $Y = C + (D - C)/\{1 + \exp[b \quad (\log(x) - \log(EC_{50}))]\}$ where C = the lower limit, D = the upper limit, b = the slope at the EC_{50}, and EC_{50} = the test solution concentration required for 50% death/paralysis of nematodes after normalizing with the control (natural death/paralysis). In the regression equation, the test 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 each test concentration and immersion period was used to calculate the EC_{50} value.

Egg hatch inhibition treatments were replicated five times, and each bioassay was performed twice. Because the ANOVA indicated no significant treatment by time interaction, means were averaged over experiments. In egg hatch inhibition bioassays, treatment means were compared using Tukey's test at $P \leq 0.05$. Statistical analysis was performed using SPSS 20.

Pot bioassays were organised in a complete randomized design with five replications and were performed twice. Since ANOVAs indicated no significant treatment by time interaction (between runs of experiment), means were averaged over experiments. The data from the pot bioassays were expressed as a percentage decrease in the number of females or galls per gram of root corrected according to the control, using the Abbott's formula: corrected percent = 100 × {1 – [females number in treated plot/females number in control plot]}. Data were fit to the log-logistic model (Seefeldt et al., 1995) to estimate the concentration that caused a 50% decrease in females and galls per gram of root (EC_{50} value). In this regression equation, the test compounds (% w/w) were the independent variables (x) and the female nematodes, or galls, (as the percentage decrease over the water control) was the dependent variable (y). Because ANOVAs indicated no significant treatment by time interaction (between runs of experiments), means were averaged over experiments. Treatments means were compared using Tukey's test at $P \leq 0.05$.

Results

J2 paralysis bioassays

Paralysis activity of *M. incognita* was more affected by *D. stramonium* and *S. nigrum* extracts than *M. javanica* (Table 1). Clear time and dose response relationships were established for *D. stramonium* and the $EC_{50/96h}$ values were 427 µg ml^{-1} for both *M. incognita* and *M. javanica* (Table 1). *Solanum nigrum* demonstrated a nematostatic effect and the mortality was stabilized 3 days post J2 immersion in test solutions.

Table 1. Efficacy of *Datura stramonium* and *Solanum nigrum* methanol extracts against *Meloidogyne incognita* and *Meloidogyne javanica*.[1]

	D. stramonium		*S. nigrum*	
Immersion period	*M. incognita*	*M. javanica*	*M. incognita*	*M. javanica*
1d	968 ± 98	>8,000	409 ± 56	686 ± 98
2d	553 ± 85	581 ± 73	507 ± 72	792 ± 95
3d	427 ± 75	427 ± 23	418 ± 78	954 ± 96

[1]Half maximal effective concentration EC_{50} ± SD (µg ml^{-1}) calculated after 1, 2, and 3 days of nematode immersion in test solutions.

Egg hatch inhibition in free eggs treated with the test compounds

The cumulative undifferentiated egg hatch was decreased significantly by both *D. stramonium* and *S. nigrum* extracts at 100 µg mL^{-1} at day 6, while in successive assessments the activity increased for *D. stramonium* and decreased for *S. nigrum* (Tables 2, 3). Concerning the percent of J2 released from eggs immersed in the two methanol extracts, again *D. stramonium* was more active since it differed from control at 10 µg ml^{-1} at day 2 while *S. nigrum* differed from the control only at concentrations equal or higher than 100 µg ml^{-1} (day 2). In the next assessment date at day 6, activity increased for *D. stramonium* and decreased for *S. nigrum* differing from control at 1 and 100 µg ml^{-1}, respectively. At day 10 the percent J2 release in control dereased naturally and thus J2 release differences among treatments were not evident thereafter (Tables 4, 5).

Inhibition of nematode development

Meloidogyne incognita densities in tomato roots and gall formation were significantly supressed when *D. stramonium* and *S. nigrum* powders were incorporated in the nematode infested soil. *Meloidogyne incognita* development in artificially inoculated tomato plants treated with the weed powders was reduced with EC_{50} values for female per gram root counts calculated for *S. nigrum* and *D. stramonium* of 1.13 and 11.40 mg g^{-1}, respectively. Galls/g root were

Table 2. Effect of *Datura stramonium* methanol extract on cumulative percent hatch of *Meloidogyne incognita* undifferentiated eggs.[1]

	D. stramonium cumulative undifferentiated egg hatch			
µg ml^{-1}	Day 2	Day 6	Day 10	Day 14
1,000	9 ± 1.0^{a}	9 ± 1.0^{a}	9 ± 1.5^{a}	9 ± 1.5^{a}
100	10 ± 5.0^{a}	10 ± 5.0^{a}	10 ± 5.0^{a}	10 ± 5.0^{a}
10	17 ± 6.5^{a}	23 ± 6.5^{ab}	23 ± 6.5^{a}	23 ± 6.5^{a}
1	14 ± 6.0^{a}	24 ± 4.5^{ab}	23 ± 6.5^{a}	23 ± 6.5^{a}
0	13 ± 8.0^{a}	35 ± 9.0^{b}	47 ± 9.0^{b}	47 ± 9.0^{b}

[1]The cumulative percent hatch of undifferentiated eggs ± SD was calculated using the formula: ((eggs$_{D0}$-eggs$_{Dx}$)/total) x 100. Eggs (20-30 per well) were collected and distributed in 24-well plates and were incubated at 27°C for 14 days in test solutions or plane water. Eggs were counted at 2, 6, 10, and 14 days post experiment establishment. Each percent data represent the mean ± SD from two experiments performed in time, with five replicates per treatment each. Values within each day were compared using Tuckey's test and those followed by different letters are significantly different at ($P \leq 0.05$).

Table 3. Effect of *Solanum nigrum* methanol extract on cumulative percent hatch of *Meloidogyne incognita* undifferentiated eggs.[1]

	S. nigrum cumulative undifferentiated egg hatch			
µg ml^{-1}	Day 2	Day 6	Day 10	Day 14
1,000	9 ± 0.5^{a}	8 ± 0.0^{a}	9 ± 6.0^{a}	9 ± 6.0^{a}
100	15 ± 2.0^{a}	16 ± 4.0^{a}	16 ± 4.0^{a}	16 ± 4.0^{a}
10	15 ± 2.0^{a}	22 ± 1.0^{ab}	27 ± 1.5^{ab}	27 ± 1.0^{ab}
1	17 ± 2.0^{ab}	24 ± 3.5^{ab}	27 ± 5.0^{ab}	27 ± 5.0^{ab}
0	25 ± 4.0^{b}	35 ± 9.0^{b}	47 ± 9.0^{b}	47 ± 9.0^{b}

[1]The cumulative percent hatch of undifferentiated eggs ± SD was calculated using the formula: ((eggs$_{D0}$-eggs$_{Dx}$)/total) x100. Eggs (20-30 per well) were collected and distributed in 24-well plates and were incubated at 27°C for 14 days in test solutions or plane water. Eggs were counted at 2, 6, 10, and 14 days post experiment establishment. Each percent data represent the mean ± SD from two experiments performed in time, with five replicates per treatment each. Values within each day were compared using Tuckey's test and those followed by different letters are significantly different at (P ≤ 0.05).

similar (Table 6), with no phytotoxicity evident at the dose range of the treatments used for the bioassay.

GC–MS analysis

The low-molecular weight polar compounds extracted from both plants *S. nigrum* and *D. stramonium* were submitted to derivatization and were chemically analyzed using GC–MS. We were able to detect amino acids, carbohydrates, carboxylic acids and some compounds with non-elucidated structures termed unknowns, from U1 to U16 (Table 7). The metabolites with the highest concentrations present in both plants were fructose, sucrose and U1 with concentrations ranging from 20 to 90 mg L^{-1}. Although sugars like glucose and galactose were abundant in *D. stramonium* aqueous extracts,

Table 4. Effect of *Datura stramonium* methanol extract on cumulative percent release of *Meloidogyne incognita* J2.[1]

	D. stramonium percent J2 release			
µg ml^{-1}	Day 2	Day 6	Day 10	Day 14
1,000	3 ± 1.5^{a}	1 ± 1.0^{a}	1 ± 1.0^{a}	1 ± 1.0^{a}
100	6 ± 4.0^{a}	5 ± 3.5^{a}	4 ± 3.0^{a}	4 ± 3.0^{a}
10	20 ± 5.5^{ab}	5 ± 5.0^{a}	4 ± 1.5^{a}	4 ± 1.5^{a}
1	31 ± 6.5^{bc}	5 ± 4.0^{a}	5 ± 3.5^{a}	5 ± 3.5^{a}
0	40 ± 5.5^{c}	20 ± 5.5^{b}	8 ± 4.5^{a}	5 ± 3.5^{a}

[1]The cumulative percent release of J2 ± SD was calculated using the formula: ((J2$_{Dx}$-J2$_{D0}$)/total)x100. Eggs (20-30 per well) were collected and distributed in 24-well plates and were incubated at 27°C for 14 days in test solutions or plane water. Released J2 were counted at 2, 6, 10, and 14 days post experiment establishment. Each percent data represent the mean ± SD from two experiments performed in time, with five replicates per treatment each. Values within each day were compared using Tuckey's test and those followed by different letters are significantly different at (P ≤ 0.05).

Table 5. Effect of *Solanum nigrum* methanol extract on cumulative percent release of *Meloidogyne incognita* J2.[1]

	S. nigrum percent J2 release			
µg ml^{-1}	Day 2	Day 6	Day 10	Day 14
1,000	2 ± 2.0^{a}	2 ± 2.0^{a}	2 ± 2.0^{a}	2 ± 2.0^{a}
100	11 ± 6.0^{ab}	5 ± 4.5^{ab}	2 ± 1.5^{a}	2 ± 1.5^{a}
10	23 ± 2.0^{bc}	6 ± 2.0^{ab}	2 ± 1.0^{a}	2 ± 1.0^{a}
1	33 ± 6.5^{c}	10 ± 5.0^{ab}	11 ± 6.5^{a}	6 ± 3.5^{a}
0	40 ± 5.5^{c}	20 ± 5.5^{b}	8 ± 4.5^{a}	±3.5^{a}

[1]The cumulative percent release of *Meloidogyne incognita* J2 ± SD was calculated using the formula: (($J2_{Dx}$-$J2_{D0}$)/total) x 100. Eggs (20–30 per well) were collected and distributed in 24-well plates and were incubated at 27°C for 14 days in test solutions or plane water. Released J2 were counted at 2, 6, 10, and 14 days post experiment establishment. Each percent data represent the mean ± SD from two experiments performed in time, with five replicates per treatment each. Values within each day were compared using Tuckey's test and those followed by different letters are significantly different at ($P \leq 0.05$).

they were not detectable in *S. nigrum* extracts. Likewise, *S. nigrum* was richer in palmitic acid and glycerol. On the other hand, the chloroform extracts of both plants showed high levels of alkaloids such as dehydrohyoscinamine, atropine, and scopolamine (Table 8). Fatty acids and monoglycerides were also present at high concentrations; for example, palmitic acid was present at 1,694 mg L^{-1} only in *S. nigrum* and monoheptadecanoate glycerol at 466 mg L^{-1} in *D. stramonium*. When the alkaloid rich extract was tested against J2, no paralysis was evidenced at the concentration range of 100 to 1,000 µg ml^{-1} (data not shown).

Discussion

A number of weeds have been studied as alternatives to synthetic nematicides. For instance, water and ethanol leaf extracts of *Euphorbia hirta, Phyllanthus amarus, Cassia obtusifolia, Sida acuta,* and *Andropogon gayanus* have been found to provoke 100% mortality on *M. incognita* juveniles at 15% to 20% (w/v) (Olabiyi et al., 2008). Chaudhary and co-workers have reported on 75% to 100% mortality of juveniles of *M. incognita* after treatment with hot water and ethanol extracts of *D. stramonium* seed at 25 to 100 mg ml^{-1}

Table 6. Efficacy of weed paste (decomposing tissues) on *Meloidogyne incognita* as calculated in pot experiments.

Females/g root			Galls/g root		
EC_{50} (mg g^{-1})	SE	95% CI	EC_{50}(mg g^{-1})	SE	95% CI
Datura stramonium					
11.40	0.92	9.48–13.32	12.85	1.19	10.39–15.33
Solanum nigrum					
1.13	0.17	0.78–1.48	1.15	0.17	0.79–1.51

SE, Standard error; CI, Confidence interval.

Table 7. Small polar metabolites extracted from *Datura stramonium* and *Solanum nigrum.*

						Concentration (mg L^{-1})	
n°	RT[a]	LRI[b]	*m/z*	Quantitative masse	Cmpd	*D. stramonium*	*S. nigrum*
1	18.303	1,099	147–233–133	147	Propanedioic acid (2TMS)	ND	48.52
2	19.498	1,166	147–205–299	147	Glycerol (3TMS)	3.61	43.36
3	19.982	1,193	147–247–129	147	U1	78.45	89.87
4	20.331	1,215	147–189–292	147	Lactic acid (2TMS)	28.29	11.70
5	20.699	1,240	147–175–117	147	U2	1.68	1.13
6	21.238	1,276	138–168–227	138	U3	7.60	ND
7	21.723	1,309	147–189–233	147	U4	6.47	2.22
8	22.473	1,361	147–233–245	147	Malic acid (2TMS)	4.90	1.70
9	22.706	1,377	147–189–219	147	U5	34.81	13.35
10	22.793	1,383	217–205–147	217	Erythrose (1MEOX) (3TMS)	ND	2.81
11	23.006	1,398	254–269–180	254	U6	–	–
12	23.161	1,410	271–169–147	147	5-hydroxymethyl 2-Furoic acid (2TMS)	14.15	ND
13	23.206	1,413	117–147–217	117	U7	–	–
14	23.243	1,416	205–292–147	147	Threonic acid (4TMS)	1.52	ND
15	23.616	1,445	147–334–245	334	U8	1.09	ND
16	24.63	1,525	103–147–217	217	Xylitol (5TMS)	1.32	1.13
17	25.339	1,583	117–147–147	117	U9	–	–
18	25.518	1,598	217–319–147	217	Altrose (5TMS)	3.96	ND
19	25.7	1,614	231–147–133	231	U10	–	–
20	25.872	1,629	246–147–129	147	U11	–	–
21	26.126	1,651	217–257–379	217	U12	–	–
22	26.462	1,679	204–379–147	204	Lyxose (1MEOX) (4TMS)	t	t
23	26.878	1,716	217–307–103	217	Arabinitol (5TMS)	2.72	2.22
24	26.889	1,717	345–255–147	345	U13	–	–
25	27.08	1,735	147–217–307	217	Fructose oxime (6TMS)	99.41	60.26
26	27.363	1,761	205–319–147	319	Glucose oxime (6TMS)	161.16	ND
27	27.562	1,779	319–205–160	319	Galactose oxime (6TMS)	43.89	ND
28	27.72	1,794	319–205–147	319	Glucitol tms	ND	18.40
29	28.313	1,851	95–83–195	95	U14	–	–
30	28.427	1,862	313–129–117	313	Palmitic acid (TMS)	ND	21.06
31	29.189	1,937	217–305–318	305	Myo-inositol (6TMS)	10.10	1.43
32	29.683	1,986	319–205–72	319	U15	–	–
33	30.64	2,087	124–361–140	124	Atropine TMS	6.04	1.23
34	31.03	2,130	98–217–330	330	Methyl-5,8-epoxyretinoate	1.87	2.34
35	31.33	2,164	59–72–126	59	Oleamide	11.28	–

36	33.11	2,387	371–147–203	371	Monopalmitin (2TMS)	4.20	2.60
37	33.73	2,436	217–289–361	217	U16	–	–
38	33.89	2,447	361–217–147	361	Sucrose (8TMS)	46.74	20.16
39	34.01	2,456	217–230–147	217	Alpha.-DL-arabinofuranoside, methyl (3TMS)	6,8658	–
40	34.54	2,491	399–217–147	399	Monostearin (2TMS)	3.21	2.06

[a]Retention time; [b]Linear retention index; ND, Not detected; TMS, trimethylsilyl; U, unknown.

Table 8. Chemical composition of chloroform extraction of *Datura stramonium* and *Solanum nigrum*.

						Concentration (mg L^{-1})	
n°	RT[a]	LRI[b]	*m/z*	Quantitative masse	Cmpd	*D. stramonium*	*S. nigrum*
1	15.30	977	77–147–174	147	U1	–	–
2	15.80	993	147–117–191	147	U2	–	–
3	17.09	1,047	130–174–188	130	U3	–	–
4	17.92	1,083	117–131–147	117	U4	–	–
5	18.55	1,113	144–218–73	144	Valine TMS	28.43	ND
6	19.22	1,150	192–191–123	192	4-methylesculetin	62.86	ND
7	19.97	1,192	109–111–183	183	U5	–	–
8	23.11	1,406	263–278–175	263	U6	–	–
9	25.77	1,620	357–299–211	299	Phosphoric acid TMS	ND	13.12
10	26.47	1,681	285–117–85	285	Myristic acid TMS	ND	51.57
11	28.32	1,852	95–96–195	95	Acetic acid, 9-methyl-9-aza-bicyclo[3.3.1] non-6-en-2-yl ester	19.97	ND
12	28.44	1,864	313–117–129	313	6-oxo-3-methoxy-n-methyl-4,5,7,8-diepoxymorphine	14.93	ND
13	28.5	1,869	117–129–313	313	Palmitic acid TMS	–	1,694.97
14	28.54	1,873	195–194–81	195	U7	–	–
15	28.95	1,913	263–294–81–67	67	Linoleic acid, methyl ester	ND	7.82
16	28.98	1,917	94–124–271	124	Dehydrohyoscinamine	–	–
17	29.47	1,966	160–262–328	328	U8	–	–
18	29.98	2,017	315–337–183	315	Lauric acid propyl ester	–	–
19	30.07	2,026	337–315–262	337	Linoleic acid TMS	169.48	3,268
20	30.15	2,035	328–262–160	328	U9	–	–
21	30.29	2,050	341–117–129	341	Stearic acid TMS	ND	834.952
22	30.68	2,091	124–361–94	124	Atropine TMS	77.95	7.00
23	30.88	2,112	328–329–160	328	U10	–	–
24	31.63	2,197	343–211–147	343	Myristic acid glycerine TMS	17.732	25.34
25	31.68	2,202	138–94–154–94	138	Scopolamine	5.65	ND
26	31.72	2,208	356–262–160	356	U11	6.38	15.27
27	32.43	2,286	356–160–444	356	U12	–	–

28	32.88	2,351	218–129–313	218	2-monopalmitin TMS	13.56	544.79
29	33.24	2,406	371–239–203	371	Palmitin TMS	–	–
30	33.87	2,446	385–147–203	385	Heptadecanoic acid glycerine TMS	466.72	ND
31	34.32	2,476	341–218–147	218	2-monostearin TMS	13.95	14.71
32	34.41	2,483	397–129–147	129	1-monooleoylglycerol TMS	–	–
33	34.48	2,487	441–399–147	441	U13	–	–
34	34.63	2,498	399–147–203	399	Stearin TMS	ND	496.04

[a]Retention time; [b]Linear retention index; ND, Not detected; TMS, trimethylsilyl; U, unknown.

(Chaudhary et al., 2013), while Elbadri and co-workers have reported high nematicidal activity levels for *D. stramonium* seed extracts on *M. incognita* J2 at 500 ppm (Elbadri et al., 2008). Herein we report on a higher *Meloidogyne* sp. paralysis activity exhibited by *S. nigrum* and *D. stramonium* methanol extracts, since the $EC_{50/3d}$ values were calculated at around 420 $\mu g\,ml^{-1}$. Interestingly the chloroform extracts of *D. stramonium* and *S. nigrum* were not active against the phytonematodes, thus suggesting the absence of activity for the alkaloids fraction. As previously demonstrated for other nematicidal plant extracts (Ntalli et al., 2013) *M. incognita* was found more susceptible than *M. javanica* when exposed to *D. stramonium* and *S. nigrum* extracts. When the weed extracts were tested for egg hatch inhibition, *D. stramonium* was more effective than *S. nigrum* in suppressing both cumulating undifferentiated egg hatch and J2 release from eggs. Similarly, water extracts of *Luffa cylindrica* and *Momordica charantia* significantly inhibited the hatching of *Meloidogyne* spp. eggs (Ononuju and Nzenwa, 2011). To the best of our knowledge this is the first report on the paralysis activity and egg hatch inhibition activity of *S. nigrum* and *D. stramonium* methanol extracts against *Meloidogyne* spp.

Interestingly when the *S. nigrum* seeds paste was used to treat nematode infested soil the EC_{50} value for reducing females per gram of tomato roots was the lowest ever reported for similar treatments by our group, namely 1.13 $mg\,g^{-1}$ (Ntalli et al., 2010; Caboni et al., 2012; Caboni et al., 2013; Aissani et al., 2015; Caboni et al., 2015). Also Radwan and co-workers have reported on *S. nigrum* powder activity on *M. incognita* but at higher concentration levels, namely 5 $g\,kg^{-1}$ (Radwan et al., 2012). It seems *S. nigrum* paste incorporated in the nematode infested soil was more active than the extract and more effective than *D. stramonium*.

Our results, on the chemical composition of the weeds under study agreed with former broad chemical screening studies (Jimoh et al., 2010). Steenkamp et al. (2004) also detected atropine and scopolamine by high performance liquid chromatography in *D. stramonium*. Additionally, linoleic acid, present at 3,268 $mg\,L^{-1}$ in *S.nigrum* was reported for its nematicidal activity on *Caenorhabditis elegans* with EC_{50} value as low as 5 $mg\,L^{-1}$ (Stadler et al., 1993). This work reports for the first time, ten metabolites in *D. stramonium* and *S. nigrum* using GC–MS after methoxylation and sylilation.

It appears that the complexity of the biological interactions among chemical constituents adds to the overall efficacy of the material. We previously found that soil incorporation of powdered plant materials had lower EC_{50} values for nematicidal activity than the respective extracts (Ntalli et al., 2010, Aissani et al., 2015, Caboni et al., 2015). Farmers do in fact utilize complex materials like waste resources, oil seed cake, and gutter oil to help manage *M. incognita* (Zhang et al., 2012) and efficacy is the sum of activities of active(s) against various nematode growth stages (egg, J2, and female laying eggs). The efficacy of the botanical nematicidals along with their side effects on non-target organisms, easiness of preparation, and cost effectiveness contribute to their overall significancy (Ntalli and Caboni, 2017). Biofumigation has been advocated as an eco-friendly tactic to manage plant-parasitic nematodes amongst which the number-one target has been *Meloidogyne* sp. (Jones et al., 2013) and Brassicas is the oldest green manure amendment for their control globally (Fourie et al., 2016). Here we prove that the production/release of nematicidal allelochemicals by *S. nigrum* when its seeds are crashed and incorporated into the soil is among the best reported (EC_{50} = 1.13 mg of *S. nigrum* powder per gram of soil) by our group. Since both *S. nigrum* and *D. stramonium* are widespread weed species, their soil incorporation could be an interesting alternative nematode control tool.

Acknowledgments

Authors are grateful to Mr. T. Koufakis and AGRIS SA for providing seeds and seedlings.

References

Abbasi, A.M., Shah, M.H., Li, T., Fu, X., Guo, X., and Liu, R.H. 2015. Ethnomedicinal values, phenolic contents and antioxidant properties of wild culinary vegetables. *Journal of Ethnopharmacology* 162: 333–45.

Aissani, N., Urgeghe, P.P., Oplos, C., Saba, M., Tocco, G., Petretto, G.L., Eloh, K., Menkissoglu-Spiroudi, U., Ntalli, N., and Caboni, P. 2015. Nematicidal activity of the volatilome of *Eruca sativa* on *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* 63: 6120–5.

Blum, U. 1996. Allelopathic interactions involving phenolic acids. *Journal of Nematology* 28: 259–67.

Byrd, D.W., Kirkpatrick, T., and Barker, K.R. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. *Journal of Nematology* 15: 142–3.

Caboni, P., Ntalli, N.G., Aissani, N., Cavoski, I., and Angioni, A. 2012. Nematicidal activity of (E,E)-2,4-decadienal and (E)-2-decenal from *Ailanthus altissima* against *Meloidogyne javanica*. 2012. *Journal of Agricultural and Food Chemistry* 60: 1146–51.

Caboni, P., Saba, M., Oplos, C., Aissani, N., Maxia, A., Menkissoglu-Spiroudi, U., Casu, L., and Ntalli, N. 2015. Nematicidal activity of furanocoumarins from parsley against *Meloidogyne* spp. *Pest Management Science* 71: 1099–105.

Caboni, P., Saba, M., Tocco, G., Casu, L., Murgia, A., Maxia, A., Menkissoglu-Spiroudi, U., and Ntalli, N. 2013. Nematicidal activity of mint aqueous extracts against the root-knot nematode *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* 61: 9784–8.

Chaudhary, K.K., Haile, A., Ayresea, Z.G., Semereab, G., and Weldegergish, T. 2013. Nematicidal activity of Eritrean weed plants against the root-knot nematode *Meloidogyne incognita*. *Nematropica* 43: 207–15.

Dhellot, J.R., Matouba, E., Maloumbi, M.G., Nzikou, J.M., Dzondo, M.G., Linder, M., Parmentier, M., and Desobry, S. 2006. Extraction and nutritional properties of *Solanum nigrum* L seed oil. *African Journal of Biotechnology* 5: 987–91.

Ding, X., Zhu, F., Yang, Y., and Li, M. 2013. Purification, antitumor activity in vitro of steroidal glycoalkaloids from black nightshade (*Solanum nigrum* L.). *Food Chemistry* 141: 1181–211.

Elbadri, G.A., Lee, D.W., Park, J.C., Yu, H.B., and Choo, H.Y. 2008. Evaluation of various plant extracts for their nematicidal efficacies against juveniles of *Meloidogyne incognita*. *Journal of Asia-Pacific Entomology* 11: 99–102.

Fourie, H., Ahuja, P., Lammers, J., and Daneel, M. 2016. Brassicacea-based management strategies as an alternative to combat nematode pests: A synopsis. *Crop Protection* 80: 21–41.

Gharbi, S., Renda, G., La Barbera, L., Amri, M., Messina, C.M., and Santulli, A. 2017. Tunisian tomato by-products, as a potential source of natural bioactive compounds. *Natural Product Research* 31: 626–31.

Giannakou, I.O. 2011. Efficacy of a formulated product containing *Quillaja saponaria* plant extracts for the control of root-knot nematodes. *European Journal of Plant Pathology* 130: 587–96.

Gouveia, M., Cordeiro, N., Teixeira, L., Abrantes, I.D.O., Pestana, M., and Rodrigues, M. 2014. *In vitro* evaluation of nematicidal properties of *Solanum sisymbriifolium* and *S. nigrum* extracts on *Pratylenchus goodeyi*. *Nematology* 16: 41–51.

Hewlett, T.E., Hewlett, E.M., and Dickson, D.W. 1997. Response of *Meloidogyne* spp., *Heterodera glycines*, and *Radopholus similis* to tannic acid. *Journal of Nematology* 29: 737–41.

Hussey, R.S., and Barker, K.R. 1973. Comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Disease Report* 57: 1025–8.

Jagtap, C.Y., Prajapati, P.K., Rudrappa, H.C., and Shukla, V.J. 2013. Pharmacognostic and phytochemical investigation of root of *Solanum nigrum* Linn.: An ethnomedicinally important herb. *International Journal of Green Pharmacy* 7: 46–9.

Jang, J.Y., Le Dang, Q., Choi, Y.H., Choi, G.J., Jang, K.S., Cha, B., Luu, N.H., and Kim, J.-C. 2015. Nematicidal activities of 4-quinolone alkaloids isolated from the aerial part of *Triumfetta grandidens* against *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* 63: 68–74.

Jimoh, F.O., Adedapo, A.A., and Afolayan, A.J. 2010. Comparison of the nutritional value and biological activities of the acetone, methanol and water extracts of the leaves of *Solanum nigrum* and *Leonotis leonorus*. *Food Chemical Toxicology* 48: 964–71.

Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K., Kikuchi, T., Manzanilla-Lopez, R., Palomares-Rius, J.E., Wesemael, W.M.L., and Perry, R.N. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14: 946–61.

Keating, K.I. 1999. Allelopathy: Principles, procedures, processes, and promises for biological control. *Advances in Agronomy* 67: 141–231.

Lee, Y.S., Park, Y.S., Nam, S., Suh, S.J., Lee, J., Kaang, B. K., and Cho, N.J. 2000. Characterization of GAR-2, a novel G protein-linked acetylcholine receptor from *Caenorhabditis elegans*. *Journal of Neurochemistry* 75: 1800–9.

Li, J., Lin, B., Wang, G., Gao, H., and Qin, M. 2012. Chemical constituents of *Datura stramonium* seeds. *Zhongguo Zhongyao Zazhi* 37: 319–22.

Li, M.H., Ding, G., Meng, Z.Q., Cai, J., and Liu, J.J. 2007. Content determination of solasonine and solamargine in *Solanum nigrum*. *Chinese Journal of Natural Medicine* 5: 360–2.

Li, W., Sun, Y.N., Yan, X.T., Yang, S.Y., Lee, S.J., Byun, H.J., Moon, C.S., Han, B.S., and Kim, Y.H. 2013. Isolation of nematicidal triterpenoid saponins from *Pulsatilla koreana* root and their activities against *Meloidogyne incognita*. *Molecules* 18: 5306–16.

Ma, L., Gu, R., Tang, L., Chen, Z.E., Di, R., and Long, C. 2015. Important poisonous plants in Tibetan ethnomedicine. *Toxins* 7: 138–55.

Mateeva, A., and Ivanova, M. 2000. Alternative methods for control of root-knot nematodes, *Meloidogyne* spp. *Acta Horticolture* 532: 109–11.

Mohy-ud-din, A., Khan, Z., Ahmad, M., Kashmiri, M.A., Yasmin, S., Asghar, M.N., and Ahmad, S.R. 2010. Epicuticular waxes from *Solanum nigrum* complex: Chemotaxonomic implications. *Asian Journal of Chemistry* 22: 2919–27.

Ntalli, N., Oplos, C., Michailidis, M., Thanasenaris, A., Kontea, D., Caboni, P., Tsiropoulos, N.T., Menkissoglu-Spiroudi, U., and Adamski, Z. 2016. Strong synergistic activity and egg hatch inhibition by (*E*,*E*)-2,4-decadienal and (*E*)-2-decenal in *Meloidogyne* species. *Journal of Pest Science* 89: 565-79.

Ntalli, N.G., and Caboni, P. 2017. A review of isothiocyanates biofumigation activity on plant parasitic nematodes. *Phytochemistry Reviews* 16 No. 5: 827–34, doi: 10.1007/s11101-017-9491-7.

Ntalli, N.G., Nasiou, E., and Menkissoglu-Spiroudi, U. 2013. Evaluation of essential oils from rosemary, orange, lavandula and false yellowhead on hatching and motility of root-knot nematode. *Journal of Agricultural Science and Technology A* 3: 603–16.

Ntalli, N.G., Vargiu, S., Menkissoglu-Spiroudi, U., and Caboni, P. 2010. Nematicidal carboxylic acids and aldehydes from *Melia azedarach* fruits. *Journal Agricultural Food Chemistry* 58: 11390–4.

Ntalli, N.G., Ferrari, F., Giannakou, I.O., and Menkissoglu-Spiroudi, U. 2011. Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and nematicidal activity of essential oils from 7 plants indigenous in Greece. *Pest Management Science* 67: 341–51.

Ntidi, K.N., Fourie, H., Mc Donald, A.H., De Waele, D., and Mienie, C.M.S. 2012. Plant-parasitic nematodes associated with weeds in subsistence agriculture in South Africa. *Nematology* 14: 875–87.

Olabiyi, T.I., Oyedunmade, E.E.A., Ibikunle, G.J., Ojo, O.A., Adesina, G.O., Adelasoye, K.A., and Ogunniran, T.A. 2008. Chemical composition and bionematicidal potential of some weed extracts on *Meloidogyne incognita* under laboratory conditions. *Journal of Plant Science and Research* 1: 30–5.

Ononuju, C.C., and Nzenwa, P.O. 2011. Nematicidal effects of some plant extracts on egg hatchability and control of *Meloidogyne* spp. in cowpea (*Vigna unguiculata* (L.)Walp. *African Journal of Plant Science* 5: 176–82.

Radwan, M.A., Abu-Elamayem, M.M., Kassem, S.M., and El-Maadawy, E.K. 2006. Soil amendment with dried weed leaves as non-chemical approach for the management of *Meloidogyne incognita* infecting tomato. *Communications in Agricultural and Applied Biological Sciences* 71: 25–32.

Radwan, M.A., Farrag, S.A.A., Abu-Elamayem, M.M., and Ahmed, N.S. 2012. Efficacy of dried seed powder of some plant species as soil amendment against *Meloidogyne incognita* (Tylenchida: *Meloidogynidae*) on tomato. *Archives of Phytopathology and Plant Protection* 45: 1246–51.

Rao, C.V., Mani, A., and Rao, P.K. 1986. Effect of plant products on egg hatch and larval mortality of *Meloidogyne incognita*. *Animal Science* 95: 397–401.

Robab, M.I., Shaikh, H., and Azam, T. 2012. Antagonistic effect of Glomus mosseae on the pathogenicity of root-knot nematode infected *Solanum nigrum*. *Crop Protection* 42: 351–5.

Sammani, A., Shammaa, E., and Chehna, F. 2013. Qualitative and quantitative steroidal alkaloids of solanum species distributed widely in Syria by TLC and HPLC. *International Journal of Pharmaceutical Science Review and Research* 23: 23–7.

Seefeldt, S.S., Jensen, J.E., and Fuerst, E.P. 1995. Log-logistic analysis of herbicide rate response relationships. *Weed Technol* 9: 218–227.

Sher, H., Aldosari, A., Ali, A., and De Boer, H.J. 2015. Indigenous knowledge of folk medicines among tribal minorities in Khyber Pakhtunkhwa, northwestern Pakistan. *Journal of Ethnopharmacology* 166: 157–67.

Stadler, M., Heidrun, A., and Olov, S. 1993. Linoleic acid-the nematicidal principle of several nematophagous fungi and its production in trap-forming submerged cultures. *Archives of Microbiology* 160: 401–5.

Steenkamp, P.A., Harding, N.M., van Heerden, F.R., and van Wyk, B.E. 2004. Fatal Datura poisoning: Identification of atropine and scopolamine by high performance liquid chromatography/photodiode array/mass spectrometry. *Forensic Science International* 145: 31–9.

Wen, Y., Meyer, S.L., Masler, E.P., Zhang, F., Liao, J., Wei, X., and Chitwood, D.J. 2013. Nematotoxicity of drupacine and a Cephalotaxus alkaloid preparation against the plant-parasitic nematodes *Meloidogyne incognita* and *Bursaphelenchus xylophilus*. *Pest Management Science* 69: 1026–33.

Whittaker, R., and Feeny, P. 1971. Allelochemics: Chemical interactions between species. *Science* 171: 757–70.

Yang, X., Wang, X., Wang, K., Su, L., Li, H., Li, R., and Shen, Q. 2015. The nematicidal effect of camellia seed cake on root-knot nematode *Meloidogyne javanica* of banana. *PLOS ONE* 10 No. 4: e0119700, https://doi.org/10.1371.

Zhang, W.P., Ruan, W.B., Deng, Y.Y., and Gao, Y.B. 2012. Potential antagonistic effects of nine natural fatty acids against *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* 60: 11631–7.

Zhou, L., Wang, J., Wang, K., Xu, J., Zhao, J., Shan, T., and Luo, C. 2012. Secondary metabolites with antinematodal activity from higher plants. Studies in natural products. *Chemistry* 37: 67–114.

Zhou, X.L., He, X.J., Zhou, G.X., Ye, W.C., and Yao, X.S. 2007. Pregnane glycosides from *Solanum nigrum*. *Journal of Asian Natural Product Research* 9: 517–23.