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Nematicidal Activity of Nonacosane-10-ol and 23a-Homostigmast-5-en-3β-ol Isolated from the Roots of *Fumaria parviflora* (Fumariaceae)

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ABSTRACT: Two bioactive nematicidal phytochemicals, viz., nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol, were isolated from the *n*-hexane fraction of the roots of *Fumaria parviflora* through activity-guided isolation. The structures of the compounds were elucidated using ^{13}C and ^{1}H nuclear magnetic resonance. Activity of the two compounds against eggs and juveniles (J2s) of *Meloidogyne incognita* was evaluated *in vitro* at the concentrations of 50, 100, 150, and 200 μg mL^{-1}. Over 120 h of incubation, the cumulative percent mortality and hatch inhibition of both of the compounds tested ranged from 20 to 100% and from 15 to 95.0%, respectively. In pot trials with tomato cultivar Riogrande, the two compounds, applied as soil drenches at the concentrations of 100, 200, and 300 mg/kg, significantly decreased the nematodes and plant growth parameters. Nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol reduced the numbers of galls (42.6 and 60.3), galling index (1.6 and 2.8), females per gram of root (37.3 and 57.0), eggs per gram of root (991.3 and 1273.0), reproduction factor (R_f) (0.1 and 0.2), and fresh root weight (14.33 and 17.0 g) at 300 mg/kg concentration and increased fresh shoot weight (49.0 and 48.4 g), dry shoot weight (28.0 and 25.3 g), and plant height (53.5 and 49.6 cm), respectively. These compounds could provide new insight in the search for novel nematicides against *M. incognita*.

KEYWORDS: *23a-Homostigmast-5-en-3β-ol, nonacosane-10-ol, Fumaria parviflora, root knot nematodes, n-hexane, nematicidal bioassay, Solanum lycopersicum*

INTRODUCTION

Root knot nematodes (*Meloidogyne* spp.) are the leading cause of crop losses in developing countries. The southern root knot nematodes (SRKNs), *Meloidogyne incognita* (Kofoid and White) Chitwood, are plant-pathogenic nematodes with a very broad host range that limits crop rotation options.[1] Yield losses caused by the root knot nematodes are generally greater in the tropical countries than those in the temperate regions because of the favorable environmental conditions in the tropics for pathogen colonization and growth, greater pathogen diversity, and a lack of financial resources to combat infestations.[2]

Fumigants, such as 1,3-dichloropropene, methyl bromide, and dazomet, and non-fumigant nervous system toxins, such as oxamyl and fenamiphos, have been used as effective nematicides to reduce the number of nematodes in the soil; however, the former is volatile. Further, the chemical treatments that target nematode nerves are a potential danger to humans.[3] Consequently, most of the nematicides, such as methyl bromide, dibromochloropropane (DBCP), and ethylene dibromide (EDB), have been banned because of health and environmental problems associated with their production and use.[4] Antagonistic crops and their naturally occurring plant products are two areas being investigated as alternatives to synthetic chemical control and have been used widely.[5] Many higher plants, herbs, and weeds are major sources of natural products used as agrochemicals, pesticides, pharmaceuticals, food additives, flavors, and fragrance ingredients. Nematicides of plant origin include thiocyanates, terpenoids, triterpenoids, steroids, phenolics, glycosides, flavonoids, essential oils, and alkaloids.[6]

Plants are capable of producing a large number of secondary metabolites, some of which have been investigated for their effects on *M. incognita*.[7] As many as 16 phytochemicals have been shown to possess inhibitory activity against the glutathione-*S*-transferase (GST) enzymes of *M. incognita*.[8] Recently, the emergence of recombinant DNA technology has opened a new field with the possibility of directly modifying the expression of genes related to metabolite biosynthesis. It is now possible to manipulate the pathways that lead to secondary plant compounds.[9] This is now well-understood that many phytochemicals (allelochemicals) possess insecticidal, hormonal, and anti-feedant activities against pests.[10]

Nematicidal phytochemicals are generally safe for humans and the environment.[11] A variety of monoterpenoids, such as borneol, carveol, citral, geraniol, and α-terpinol, have shown incredible nematicidal activity against *M. incognita*.[12] The suppressive effect of many plant extracts, for example, marigold (*Tagetes erecta*) and neem (*Azadirachta indica*), on SRKNs has been demonstrated in a number of reports.[13,14] Nematicidal phytochemicals isolated from the plants in the family Asteraceae, such as α-terthienyl isolated from *Tagetes* spp., proved effective in an *in vitro* study; however, these phytochemicals were not very promising in the soil. Another

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group of phytochemicals, i.e., "polyacetylenes", isolated from Asteraceae have shown relatively better performance against *M. incognita* and *Pratylenchus penetrans*.[15] Unfortunately, none of these compounds or the crude extracts have been developed into commercial formulations.

Previously, in a screening for nematicidal activity, we found that the roots and stem *n*-hexane extracts of *Fumaria parviflora* Lam (Fumariaceae) at a concentration of 50.0 mg mL^{-1} showed the strongest nematicidal activity against juveniles (J2s) and eggs of *M. incognita*, while the same extracts at 3000 mg/kg concentration significantly curbed the pathogen in tomato roots in an *in planta* study.[16] Moreover, different classes of bioactive constituents, viz., alkaloids, flavonoids, glycosides, tannins, saponins, steroids, and phenols, were detected in the roots and stem extracts of *F. parviflora*.[16]

F. parviflora, commonly known as fumitory, locally known as papra or pitpapra in Pakistan, is native to Asia, Africa, and Europe.[17] A number of isoquinoline alkaloids, including protopine, protoberberine, benzylisoquinoline, steroids, and fatty acids, have been isolated from the plant.[18−20] Although relatively few studies have been performed on the pure compounds, no bioactive nematicidal compounds against root knot nematodes and other plant parasitic nematodes have been isolated and reported from this plant. In the present study, we report (a) the isolation and structure elucidation of nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol from the *n*-hexane root extracts of *F. parviflora* and (b) *in vitro* nematicidal activity on egg hatching and J2 viability and *in planta* nematicidal effect of the two compounds on *M. incognita* in tomato under artificially inoculated screen house conditions.

MATERIALS AND METHODS

Chemicals. All commercial-grade solvents, viz., *n*-hexane, ethyl acetate (EtOAC), and methanol (MeOH), were used after distillation. Dimethyl sulfoxide (DMSO, 99.9%, Merck, Darmstadt, Germany) was used for dissolving the dried extracts. For column chromatography (CC), laboratory-grade chemicals (Merck, Germany) were used.

Plant Material, Extraction, Isolation, and Identification of Nematicidal Compounds. Mature *F. parviflora* plants were collected in the months of March and April of 2009 from the wheat fields of New Agricultural Research Farm, Malakandher, The University of Agriculture, Peshawar, Pakistan. The plants were identified, and a voucher specimen (ISH-1732) was deposited in the herbarium of the Department of Botany, University of Peshawar, Peshawar, Pakistan. The air-dried, powdered roots (1 kg) of the plant were extracted with *n*-hexane (2 × 1000 mL) for 4 h in a Soxhlet apparatus. The extracts were concentrated on a rotary evaporator under vacuum to yield the crude residue (38.0 g). The crude residue was subjected to silica gel CC. Elution was carried out using a *n*-hexane/ethyl acetate mixture (100:0, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100, v/v) to give 11 major fractions (F1−F11). The F3 and F4 fractions were further subjected to CC using *n*-hexane/ethyl acetate (1:17 and 1:19, v/v) yielding two compounds (45.0 and 29.0 mg), and their structures were determined by spectroscopic methods.

General Techniques Employed for the Purification of Compounds. All extracts were filtered through Whatman No. 1 (11 μm) filter paper (GE Healthcare, U.K.) and evaporated to dryness at 60 °C under reduced pressure using a rotary evaporator (BUCHI, Rotavapor, U.K.). CC was carried out using silica gel (Si 60, 70−240 mesh, E. Merck) as the stationary phase and organic solvents (*n*-hexane, EtOAC, and MeOH) as the mobile phase. The extracts, fractions, and pure compounds were checked by thin-layer chromatography (TLC) using Merck Kieselgel 60 F254 precoated silica gel (20 × 20 cm, 10 μm pore size, Merck, Germany) as the stationary phase and *n*-hexane/chloroform as the mobile phase. The black dense and compact spot of both of the compounds were detected after spraying the TLC plate with ceric sulfate solution (10% H_2SO_4). Melting points of the compounds were determined in glass capillary tubes using the melting point apparatus by Bibby Scientific Limited, Stone, U.K., catalog number SMP10, and were not corrected. Electron impact mass spectrometry (EIMS) was performed by JEOL MSRoute using a direct insertion probe. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on Bruker AVANCE 400.

Morphological Identification of *Meloidogyne* Species and Nematicidal Bioassay. Identification of the root knot nematodes was carried out on the basis of the perineal pattern morphology. Mature females from large galls on the roots of tomato plants were dissected. Perineal patterns (10−15) from each sample were mounted in glycerin. Glycerin-infiltrated specimens were examined under a compound microscope with oil immersion to study their characteristics.[21]

In vitro nematicidal bioassays were performed using a 24 microwell plate (Multiwell 24 well, Becton Dickinson, Franklin Lakes, NJ). A pure culture of *M. incognita* was maintained via a single egg mass inoculation on susceptible tomato cultivar Riogrande, grown in the screen house of the Plant Pathology Department, The University of Agriculture, Peshawar, Pakistan, for 60 days. Nematode eggs were extracted with 1% NaOCl solutions following a standard protocol.[22] Eggs were rinsed with distilled water using a 25 μm aperture sieve, and J2s were obtained using surface-sterilized eggs, which hatched after 3−4 days.[16] The nematicidal activity of the two compounds (23a-homostigmast-5-en-3β-ol and nonacosane-10-ol) against both the J2s and eggs of *M. incognita* was determined in separate and simultaneous experiments using a microwell bioassay. Stock solutions of the compounds were prepared in 1% DMSO, which was further diluted with distilled water. The final concentration of the DMSO did not exceed 1% (v/v). Eggs (approximately 1000 ± 50) and J2s (200) were transferred to each well of a 24 microwell plate in a final volume of 1 mL at a final concentration of 50, 100, 150, and 200 μg mL^{-1} of each of the compounds.[16] Distilled water with DMSO (1%, v/v) was used as a negative control. Five repetitions for each concentration along with the control were performed, and both experiments were performed twice at room temperature (25 °C). Finally, the plates were incubated at room temperature for 5 days in the dark. The total numbers of unhatched eggs or active/inactive J2s in each well were counted after 24, 48, 72, 96, and 120 h of incubation. After the last count, eggs and J2s solutions of *M. incognita* were maintained in distilled water to observe hatching or mobility in water after 24 h. Juveniles were defined as dead if they did not move, even after mechanical touching.[23] The percentage of unhatched eggs and the J2 mortality was calculated for each well, and the results of separate experiments were subjected to statistical analysis.

***In Planta* Experiments.** The pot experiment was carried out in the greenhouse (±25 °C) of the Plant Pathology, The University of Agriculture, Peshawar, Pakistan, during spring and autumn of 2010. Air-dried and steam-sterilized sand/clay-loam soil (1:1, v/v) was placed in 15 cm clay pots (1 kg of soil per pot). Eggs of *M. incognita* were extracted from the 3-month-old tomato seedlings maintained in a greenhouse. Eggs + J2s (about 5000 ± 10) were applied to the roots of 14-day-old tomato seedlings using a sterilized micropipet. Three concentrations (100, 200, and 300 mg/kg) of 23a-homostigmast-5-en-3β-ol and nonacosane-10-ol were first dissolved in DMSO (1%, v/v) using stock solutions (29.0 and 45.0 mg mL^{-1}, respectively) of the two compounds. Each compound at a rate of 50 mL per pot was applied as a root drench application, at 2 days after inoculation. Plants treated with only distilled water (45 mL) in 5 mL of DMSO (1%, v/v) served as the control. Pots were fertilized weekly with 100 mL of 0.1%, 20-5-32 + micronutrients hydro-sol fertilizer (Engro Crop Ltd., Peshawar, Pakistan) and watered 3 times a week with fresh water (350 mL of water per pot). The experiment was laid out in a completely randomized (CR) design with five replications (each replicate consisting of a single potted plant) and terminated 60 days after inoculation when plants were fully developed and showing symptoms (yellowing and stunting). The number of galls, root galling index (GI),

number of females per gram of root, number of eggs per gram of root, and reproduction factor (R_f) were assessed. For the GI, a 0−5 galling scale was used (where 0 is no gall on roots, 1 is 1−2 galls per root, 2 is 3−10 galls per root, 3 is 11−30 galls per root, 4 is 31−100 galls per root, and 5 is more than 100 galls per root).[24] Eggs were extracted from 1 g of roots, and the R_f value (final nematode numbers per plant P_f/initial nematode numbers per plant P_i) was calculated.[24]

Statistical Analysis. Data were subjected to analysis of variance (ANOVA) using Statistix (NH Analytical Software, Roseville, MN). In the hatching and mortality J2s experiments, for each treatment and repetition, the area under cumulative number of nematodes percent hatch inhibition (AUCPHI) and area under cumulative number of nematodes percent mortality (AUCPM) were estimated by trapezoidal integration.[25] Treatment means of AUCPHI and AUCPM were compared using Fisher's protected least significant difference (LSD) test at $p = 0.05$. In the *in vivo* experiment, the data were analyzed by ANOVA. Treatment means of the different parameters were compared using Fisher's protected LSD test at $p = 0.05$.[26]

RESULTS

Bioactive Nematicidal Compounds and Their Characterization. Bioactive nematicidal compounds, viz., nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol, were isolated on the basis of bioactivity-directed fractionation. TLC of both of the compounds showed R_f values of 0.65 and 0.32 for nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol, respectively. Structure elucidation of the two compounds was performed by spectroscopic methods and its comparison to the literature values. The structures of compounds are given in Figure 1.

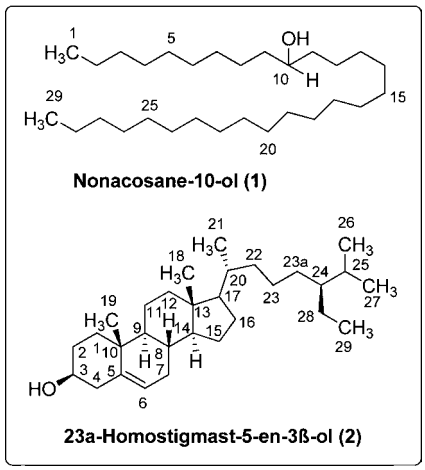

Figure 1. Structure of nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol isolated from the root *n*-hexane fraction of *F. parviflora*.

Nonacosane-10-ol (1) (Figure 1). White amorphous powder (*n*-hexane). melting point (mp) 83−84 °C (literature mp, 81−82 °C).[27] Molecular formula $C_{29}H_{60}O$, deduced from electron impact mass spectrometry (EIMS) *m/z*: 424. IR $\overline{\upsilon}_{max}$ (cm^{-1}): 3318.1 (OH st), 2953.9 and 2915.4 (CH st). ^{1}H NMR δ: 3.561 (1H, br s, H-10), 1.409 (4H, br s, H-9 and H-11), 1.235 (48H, s, H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21, H-22, H-23, H-24, H-25, H-26, H-27, and H-28), 0.860 (6H, t, J = 6.5 Hz, H-1 and H-29). ^{13}C NMR δ: 72.03 (C-10), 37.50 (C-9 and C-11), 31.93 (C-3 and C-27), 31.90 (C-7 and C-13), 29.70 (C-5, C-6, C-14, C-15, C-16, C-27, C-18, C-19, C-20, C-21, C-22, C-23, C-24, and C-25), 29.32 (C-4 and C-26), 25.66 (C-8 and C-12), 14.10 (C-1 and C-29). On the basis of the above experimental data and a comparison to literature data, the compound was identified as nonacosane-10-ol.

Homostigmast-5-en-3β-ol (2) (Figure 1). White amorphous powder ($CHCl_3$). mp 125−127 °C (literature mp, 125−127 °C).[28] Molecular formula $C_{30}H_{52}O$, deduced from the EIMS *m/z*: 428.4. IR $\overline{\upsilon}_{max}$ (cm^{-1}): 3421.1 (OH st), 2917.3 and 2848.8 (CH st). Ultraviolet (UV) spectra showed absorbance at 280 nm. ^{1}H NMR δ: 5.34 (1H, br d, J = 4.8 Hz, H-6), 3.50 (1H, septet, H-3), 2.25 (2H, m, H-4), 1.98 (1H, m, H-8), 1.93 (1H, m, H-7), 1.84 (1H, m, H-7), 1.81 (1H, m, H-1), 1.81 (1H, m, H-25), 1.54 (1H, m, H-15), 1.52 (2H, m, H-2), 1.52 (2H, m, H-23), 1.51 (1H, m, H-22), 1.45 (2H, m, H-11), 1.43 (1H, m, H-20), 1.23 (2H, m, H-28), 1.16 (2H, m, H-16), 1.14 (2H, m, H-12), 1.12 (2H, m, H-23a), 1.10 (1H, m, H-15), 1.10 (1H, m, H-17), 1.06 (1H, m, H-1), 1.06 (1H, m, H-14), 0.99 (3H, d, J = 8.0 Hz, H-21), 0.99 (1H, m, H-22), 0.91 (1H, m, H-9), 0.91 (1H, m, H-24), 0.86 (3H, t, J = 7.0 Hz, H-29), 0.83 (3H, d, J = 6.8 Hz, H-27), 0.80 (3H, s, H-19), 0.79 (3H, d, J = 6.8 Hz, H-26), 0.75 (3H, s, H-18). ^{13}C NMR δ: 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 55.0 (C-17), 50.2 (C-9), 45.9 (C-24), 42.3 (C-4), 42.3 (C-13), 39.8 (C-12), 36.5 (C-10), 37.3 (C-1), 36.2 (C-20), 34.0 (C-22), 31.9 (C-2), 31.9 (C-8), 31.7 (C-7), 23.1 (C-28), 26.1 (C-23a), 29.7 (C-23), 29.3 (C-25), 28.2 (C-16), 24.4 (C-15), 21.1 (C-11), 19.8 (C-27), 19.4 (C-19), 19.0 (C-26), 18.8 (C-21), 12.1 (C-29), 12.0 (C-18). On the basis of the above experimental data, the compound was identified as homostigmast-5-en-3β-ol.

Perineal Pattern Morphology. *M. incognita* was identified by perineal pattern morphology of the root knot nematode. Observations under an Olympus compound microscope (100×) revealed that the characteristic lateral lines were absent in *M. incognita* and a squarish, high dorsal arch containing a distinct whorl around the tail terminus was the most conspicuous diagnostic feature of this perineal pattern.

Effect of Nonacosane-10-ol and 23a-Homostigmast-5-en-3β-ol on Hatching of *M. incognita*. Percent hatch inhibition of *M. incognita* and AUCPHI were significantly increased (p = 0·05) when exposed to different concentrations (50, 100, 150, and 200 μg mL^{-1}) of nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol with respect to distilled water with DMSO (as a control) (panels a and b of Figure 2). Differences among all four concentrations were found significant using Fisher's protected LSD test. Final cumulative hatch inhibition ranged from 20 to 100% for both compounds (panels c and d of Figure 2), whereas the highest hatch inhibition of 95.0 and 90.3% was observed at a concentration of 200 μg mL^{-1} for nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol, respectively (panels c and d of Figure 2). The final cumulative percent hatch (R^2 = 0.96 and 0.97; panels c and d of Figure 2) and AUCPHI (R^2 = 0.95 and 0.99; panels e and f of Figure 2) showed a polynomial type of increase with an increase in the compound concentration.

***In Vitro* Nematicidal Activity of Nonacosane-10-ol and 23a-Homostigmast-5-en-3β-ol on J2s Mortality of *M. incognita*.** Mortality of J2s of *M. incognita* significantly increased (p = 0.05) over time by all four concentrations of the active compounds throughout the experiment (panels a and b of Figure 3). Final J2s mortality and AUCPM of both compounds were significantly lower in J2s incubated in distilled water (as a control) than in those incubated at 50, 100, 150, and 200 μg mL^{-1} concentrations of the compounds (panels a and b of Figure 3). All of the concentrations showed significant differences using Fisher's protected LSD test. Final cumulative

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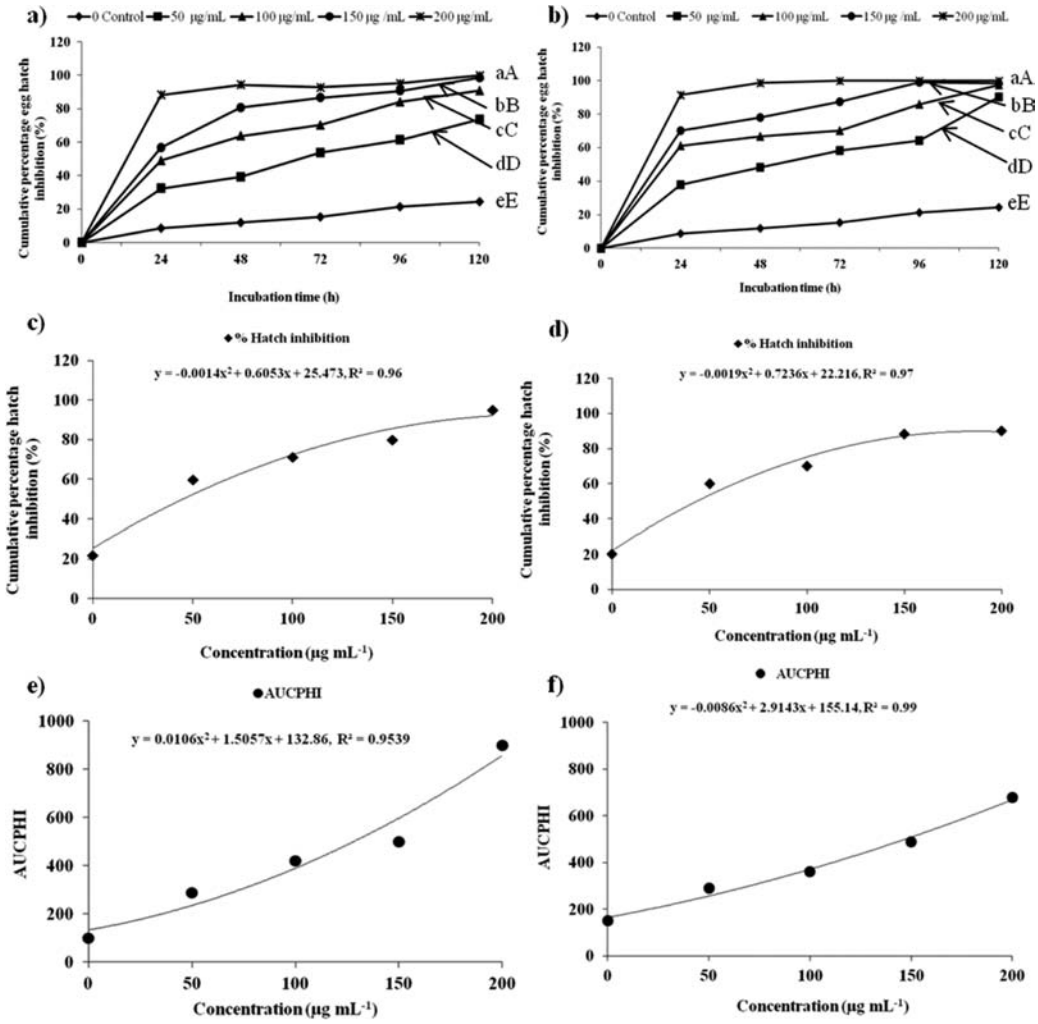

Figure 2. Cumulative hatch inhibition of *M. incognita* using (a) nonacosane-10-ol and (b) 23a-homostigmast-5-en-3β-ol over 120 h of incubation at 25 °C over a series of concentrations of the pure compounds. Each point represents the average of two experiments with five replicates. AUCPHI of curves from each treatment combination followed by the same lowercase (first experiment) or uppercase (second experiment) letter do not differ significantly ($p > 0.05$) according to Fisher's protected LSD test. Relationship between pure compound concentrations (50, 100, 150, and 200 μg mL^{-1}) and final cumulative hatch (panels c and e for nonac-sane-10-ol) and AUCPHI (panels d and f for 23a-homostigmast-5-en-3β-ol) of *M. incognita*. The lines represent the predicted function expanded by the exponential model.

percent mortality reached 100% for both of the compounds when J2s were exposed to a concentration of 200 μg mL^{-1} (R^2 = 0.98 and 0.98; panels c and d of Figure 3). The exposure period and increase in the concentration of the compounds directly increased the J2s mortality and AUCPM (R^2 = 0.99 and 0.98; panels e and f of Figure 3).

***In Planta* Experiment.** Significant differences ($p < 0.05$) among the three different concentrations (viz., 100, 200, and 300 mg/kg) of the two compounds in comparison to the control (H_2O + DMSO) were recorded (Table 1). Both compounds at all concentrations significantly ($p < 0.05$) affected all of the nematode parameters measured, showing a substantial decrease in the nematode parameters (Table 1). Nonacosan-10-ol showed the strongest nematicidal effects at all of the tested concentrations. Number of galls, GI, number of females per gram of root, number of eggs per gram of root, and R_f markedly decreased, whereas fresh shoot weight (18.4 and 12.4%), dry shoot weight (34.4 and 18.3%), and plant height (21.6 and 14.8%) increased over the untreated control even at the lowest concentration (100 mg/kg) of nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol, respectively. All of the concentrations tested showed no phytotoxic effect on the plants.

DISCUSSION

Previously, we have reported that the roots and stem extracts of *F. parviflora* prepared in four different solvent systems (*n*-hexane, ethyl acetate, chloroform, and methanol) had a strong nematicidal effect on egg hatching and J2s of *M. incognita* at 50.0 mg mL^{-1} and 3000 mg/kg concentration in *in vitro* and *in planta* studies, respectively.[16] The hatch inhibition and J2s mortality of *M. incognita* were greatly increased (100%) by the *n*-hexane root extracts compared to all of the other extracts.[16] In the present study, the *n*-hexane root extract of *F. parviflora* was subjected to CC using *n*-hexane/ethyl acetate to give two compounds, viz., nonacosan-10-ol and 23a-homostigmast-5-en-3β-ol, and their *in vitro* and *in vivo* nematicidal activities were evaluated. The suppressive effects of some phytochemicals on the nematode population have been well-documented in several pathosystems.[11] The present study clearly demonstrated that *in vitro* egg hatch inhibition and J2s mortality increased, whereas nematode parameters, such as the number of galls, GI, females

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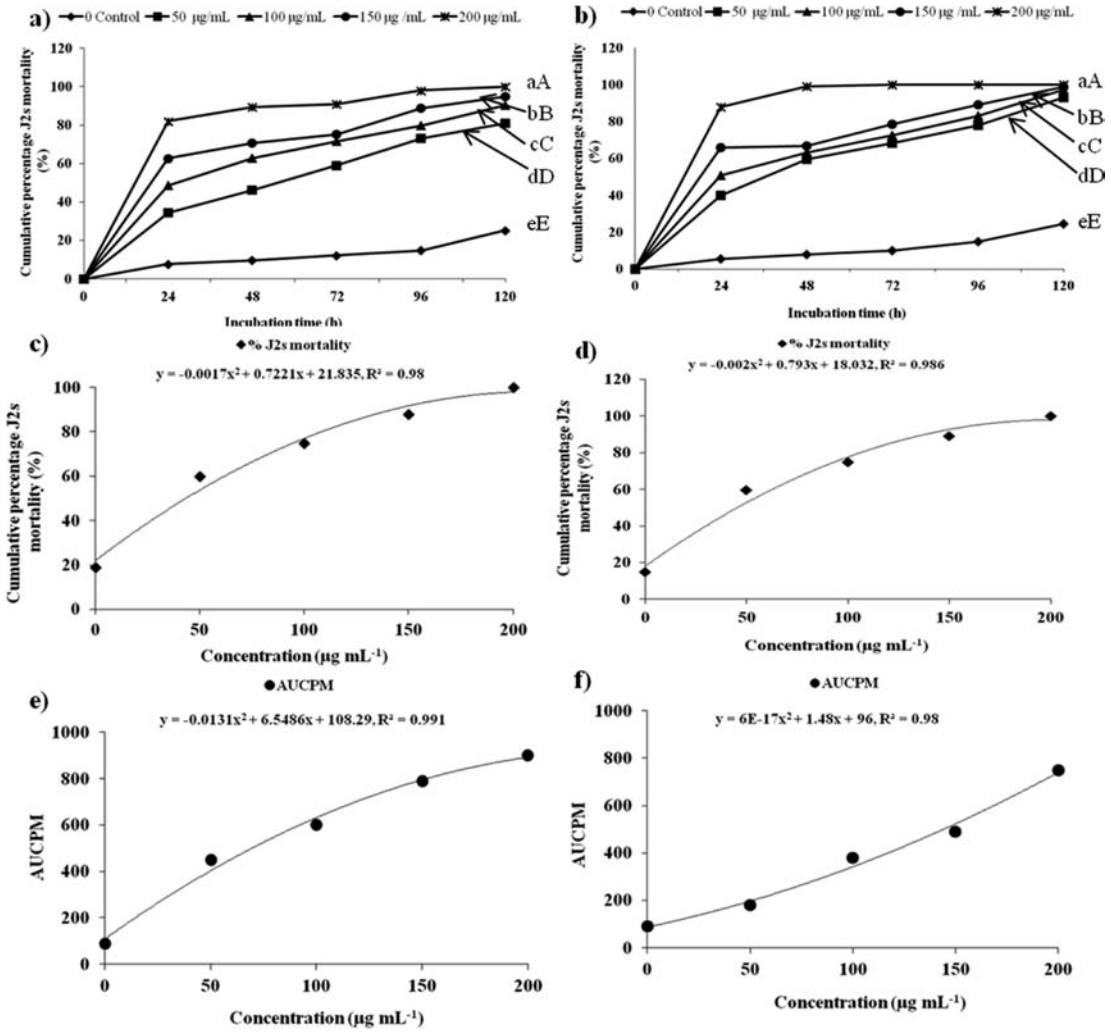

Figure 3. Cumulative percent mortality of *M. incognita* using (a) nonacosane-10-ol and (b) 23a-homostigmast-5-en-3β-ol over 120 h of incubation at 25 °C over a series of concentrations of the pure compounds. Each point represents the average of two experiments with five replicates. AUCPM of curves from each treatment combination followed by the same lowercase (first experiment) or uppercase (second experiment) letter do not differ significantly ($p > 0.05$) according to Fisher's protected LSD test. Relationship between pure compound concentrations (50, 100, 150, and 200 μg mL^{-1}) and final cumulative percent mortality (panels c and e for nonac-sane-10-ol) and AUCPM (panels d and f for 23a-homostigmast-5-en-3β-ol) of *M. incognita*. The lines represent the predicted function expanded by the polynomial model.

per gram of root, and R_f in the screen house, were reduced markedly and plant growth parameters, viz., fresh shoot weight, dry shoot weight, fresh root weight, and plant height, were enhanced by increasing the concentrations of the two tested compounds with no known phytotoxicity symptoms. An increase in the plant weight and height was due to the ability of these compounds in suppressing the activity of nematodes at the rootzone, thereby allowing the plants to flourish, hence the higher dry matter accumulation.

Nonacosane-10-ol has been identified within several essential oils.[29] It has been reported to be an integral part of a pheromone of *Orgyia leucostigma*, and evidence suggested that it played a role in the chemical communication of several insects, including the female *Anopheles stephensi* mosquito.[30] The ^{1}H and ^{13}C NMR spectral data of 23a-homostigmast-5-en-3β-ol was in close agreement with that of β-sitosterol.[28] In ^{13}C NMR, the difference is of an extra CH_2 at δ 29.7 (CH_2-23a). Both, the β-sitosterol and stigmasterol as well as campesterol have been reported from *F. parviflora* and other members of Fumariaceae.[31]

To the best of our knowledge, the two compounds have not been previously investigated for their *in vitro* and specifically *in planta* nematicidal activity, except the β-sitosterol, which has only been tested in a preliminary *in vitro* study.[32] In the present study, we reported egg hatch inhibition of 95.0 and 90.3% by nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol, respectively, at the highest concentration of 200 μg mL^{-1}, whereas J2s mortality of 100% was found for both the compounds at the same concentration. A dose-dependent effect was also observed when increasing the concentration of both of the compounds evaluated in the *in vitro* and *in planta* studies. A significant reduction in egg hatching and percent J2s viability was observed at all of the concentrations; however, the 200 μg mL^{-1} concentration was highly effective for the two compounds. Our findings of nematicidal activity of Fumariaceae agree with the results of the other researchers, who found 59.0% J2s mortality of *Meloidogyne javanica* using ethanol extracts of *Fumaria indica* at 1000 mg/kg concentration.[33] In the current study, an alcohol (nonacosane-10-ol) has been found effective in suppressing nematodes in the *in vitro* study causing 100% J2s mortality and reducing GI to 1.6 in *in planta* studies. An

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Table 1. Nematicidal Effects of Different Concentrations of Nonacosane-10-ol and 23a-Homostigmast-5-en-3β-ol from the *n*-Hexane Root Extracts of *F. parviflora* on *M. incognita* and Plant Growth Parameters of Tomato under Screen House Conditions[a]

mg/kg[b]	number of galls	GI[c]	females per gram of root	eggs per gram of root	R_f (P_f/P_i)[d]	fresh shoot weight (g)	dry shoot weight (g)	plant height (cm)	fresh root weight (g)
				Nonacosan-10-ol					
0 (H_2O)	104.0 a	5.0 a	71.0 a	6750.0 a	1.4 a	38.5 d	18.6 d	39.7 f	25.5 a
100	81.6 bc	3.6 b	58.3 b	1336.7 d	0.3 d	45.6 b	25.0 b	48.3 cd	17.3 bc
200	64.0 d	3.0 bc	49.0 c	1145.5 d	0.2 e	47.3 ab	26.0 b	49.7 b	15.6 cd
300	42.6 e	1.6 d	37.3 d	991.3 f	0.1 f	49.0 a	28.0 a	53.5 a	14.3 d
				23a-Homostigmast-5-en-3β-ol					
0 (H_2O)	104.0 a	5.0 a	71.0 a	6750.0 a	1.4 a	38.5 d	18.6 d	39.7 f	25.5 a
100	86.0 b	3.8 b	68.3 a	2133.3 b	0.4 b	43.3 c	22.0 c	46.6 e	19.6 b
200	75.0 c	3.3 bc	60.3 b	1773.3 c	0.3 c	46.3 b	23.3 c	48.0 de	18.6 b
300	60.3 d	2.8 c	57.0 b	1273.3 c	0.2 d	48.4 d	25.3 b	49.6 bc	17.0 b
LSD value ($p \leq 0.05$)[e]	8.8	0.7	7.9	127.1	0.02	1.6	1.5	1.4	2.8

[a]Data are means of 10 replicated plants per treatment using the combination of two experiments. Means followed by the same letter do no differ significantly ($p \geq 0.05$) according to the Fisher's protected LSD test. [b]The 0 mg/kg treatment was H_2O (control) mixed with DMSO (1%, v/v). [c]GI: 0 is no gall on roots; 1 is 1−2 galls per root; 2 is 3−10 galls per root; 3 is 11−30 galls per root; 4 is 31−100 galls per root; and 5 is more than 100 galls per root. [d]R_f = final nematode numbers per plant (P_f)/initial nematode numbers per plant (P_i). [e]LSD value at $\alpha = 0.05$.

aliphatic alcohol, viz., 1-octanol, isolated from *Allium grayi* (Liliacea) has shown toxicity toward *M. incognita* juveniles.[34] Plant-derived sterols have also shown toxicity toward nematodes; for example, 74.4% mortality against J2s of *M. incognita* has been reported when β-sitosterol and stigmasterol were applied together at 5.0 μg mL^{-1}.[32] The nematicidal activity of the two compounds (nonacosan-10-ol and 23a-homostigmast-5-en-3β-ol) was further strengthened by the data obtained from the *in vivo* study. In the greenhouse study, nonacosan-10-ol and 23a-homostigmast-5-en-3β-ol evaluated at 100, 200, and 300 mg/kg concentrations significantly decreased the number of galls, GI, female nematodes per gram of root, eggs per gram of root, and R_f, whereas the fresh and dry shoot weights and the plant height showed a significant increase over the untreated control. The two compounds directly affected nematode activity by interfering with the nematode hatching and J2s mortality, as reported for other phytochemicals.[11] However, nonacosane-10-ol was more effective in terms of nematicidal activity than the sterol at all of the tested concentrations in *in vitro* and *in planta* studies. It has been reported that alcohols may be involved in the nematode suppression of velvet beans (*Mucuna pruriens*, Fabaceae), which have gained popularity as a cover or green manure crop. Velvet bean extract yielded 1-triacontanol and triacontanyl tetracosanoate, and both of the compounds inhibited the hatching of *M. incognita* at 1.0%.[34]

The highest activity of nonacosane-10-ol against J2s and eggs of *M. incognita* could be attributed to the presence of the OH group at C-10 coupled with the long straight chain, and the highest activity of 23a-homostigmast-5-en-3β-ol against J2s and eggs of *M. incognita* could be attributed to the presence of the OH group at C-3. These results agreed with the previous findings that alcohols, phenols, and aldehydes were more reactive against *Bursaphelenchus xylophilus*,[23] whereas an aliphatic ketone (2-undecanone) was found highly effective against *M. incognita* (EC_{50} = 20.6) and *M. javanica* (22.5 mg/L).[35] The nematicidal activities of the compounds vary with differences among the functional groups, saturation, and carbon skeleton.[36] In a test with alkanals and 2*E*-alkenols, compounds with C_8−C_{11} chain lengths showed 100% nematicidal activity against the pine wood nematode (*B. xylophilus*) at 0.5 mg mL^{-1} concentration.[37] Many research findings revealed that nematicidal activity of the compound with the hydroxyl group (OH) or the methoxy group (OCH_3) has shown better activity than the acetyl group.[38]

The current results suggested that noncosane-10-ol and 23a-homostigmast-5-en-3β-ol could be useful nematicides for *M. incognita* and would be novel alternatives to the presently used toxic synthetic nematicides, and these emerging nematicides and biopesticides may provide valuable new components within future integrated management strategies for root knot nematodes.

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Notes

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