

Dose–response effects of clove oil from *Syzygium aromaticum* on the root-knot nematode *Meloidogyne incognita*[†]

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Abstract

BACKGROUND: Clove oil, derived from the plant *Syzygium aromaticum* (L.) Merr. & Perry, is active against various organisms, and was prepared in a soy lecithin/detergent formulation to determine concentrations active against the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood.

RESULTS: In microwell assays, the mean effective clove oil concentration that reduced egg hatch by 50% (EC₅₀) was 0.097% (v/v) clove oil; the EC₅₀ for second-stage juvenile (J2) viability was 0.145% clove oil (compared with carrier control treatments). Volatiles from 5.0% clove oil reduced nematode egg hatch in water by 30%, and decreased viability of hatched J2 by as much as 100%. Reductions were not as large with nematodes in carrier. In soil trials with J2 recovered from Baermann funnels, the EC₅₀ = 0.192% clove oil (compared with water controls).

CONCLUSION: The results demonstrated that the tested formulation is active against *M. incognita* eggs and J2, that the EC₅₀ values for J2 in the microwell studies and the soil recovery tests were similar to each other and that direct contact with the clove oil is needed for optimal management results with this natural product.

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Keywords: clove oil; *Meloidogyne incognita*; natural product; nematicide; nematode; phytochemical

1 INTRODUCTION

Conventional chemical nematicides used in agricultural production may be deleterious to the environment and to non-target organisms. Consequently, alternatives such as biorationals are being investigated for use against plant-parasitic nematodes. One group of plant-derived compounds, the essential oils, have demonstrated activity against multiple nematode taxa and are traditionally used in folk medicines as anthelmintic agents.^{1–6} Since these extracts are biodegradable and are potentially suitable for integrated disease management programs, they are being considered as nematode management agents.

Clove oil, which is derived from the buds, stems, leaves and/or flowers of the clove plant *Syzygium aromaticum* (L.) Merr. & Perry (= *Eugenia caryophyllata* Thunb.), is active against a number of organisms, including microbes, insects, nematodes and plants.^{7–16} Examples of pathogenic microbes that are affected by clove oil, or by its main constituent

eugenol, are bacteria such as *Erwinia carotovora* (Jones) Bergey *et al.*, *Escherichia coli* (Migula) Castellani & Chalmers, *Staphylococcus* spp. Rosenbach and *Helicobacter pylori* (Marshall *et al.*) Goodwin *et al.*^{17–19} In experimental use for management of soilborne plant diseases, a clove oil formulation was effective for controlling the plant-pathogenic fungi *Fusarium oxysporum* Schltdl.:Fr. and *Phytophthora nicotianae* Breda de Haan,^{20,21} and clove oil has been patented as a plant-derived fungicide.²²

Clove oil is also active against plant-parasitic nematodes.^{13,23–26} Application of clove oil as a nematicide would enhance the use of this compound for management of soilborne plant pathogens by increasing the spectrum of target plant parasites. Consequently, this study was undertaken to test a soy lecithin/detergent formulation of clove bud oil for activity against the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. Microwell tests were conducted to generate dose–response

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curves and calculate effective concentrations of clove oil required to kill 50% of the second-stage juveniles (J2) populations or reduce egg hatch by 50% (EC₅₀ values), and to investigate activity of volatiles from the essential oil. In addition, soil studies were conducted to determine whether J2 would be affected by the clove oil formulation utilized in the microwell experiments.

2 MATERIALS AND METHODS

2.1 Preparation of clove oil and of *Meloidogyne incognita*

Clove oil (Sigma-Aldrich, MO), made from clove buds, was emulsified in an aqueous carrier solution of 0.25% L- α -phosphatidylcholine (lecithin) from soybean (Sigma-Aldrich) and a non-ionic surfactant Triton X-114 (Rohm & Haas, PA) (0.1%) by vortexing the constituents for at least 2 min in a Thermolyne Maxi Mix II type vortexer (Barnstead International, IA).^{27,28}

Race 1 of *M. incognita*, originally isolated in Maryland, was grown on pepper (*Capsicum annuum*) 'PA-136' in greenhouse pots. Surface-sterilized eggs and J2 for assays were prepared similarly to previously described procedures.²⁹ To summarize, egg masses from plant roots were rinsed 3 times with sterile distilled water (DI), the eggs were released from the egg masses and surface-sterilized by agitation for 3.5 min in 5 g L⁻¹ sodium hypochlorite, debris was allowed to settle for 0.5 min and the eggs were removed and refrigerated overnight at 7 °C and used the next day for assays. Additional sterilized eggs were placed on a Spectra/Mesh Nylon Filter (Spectrum Laboratories Inc., CA) with 30 μ m diameter openings in an autoclaved storage dish, and J2 that passed through the filter within 72 h were collected and used immediately for assays.

2.2 Microwell assays of clove oil activity against *Meloidogyne incognita*

For the microwell assays, nematodes were pipetted into 24-well cell culture plates (Costar; Corning Incorporated, NY), with 0.9 mL treatment and 0.1 mL nematode inoculum in sterile distilled water per well.²⁹ Approximately 100 eggs were added to each well to determine effects on egg hatch; to assay for effects on J2, 50 J2 were placed into each well. Five wells were used per treatment per assay, and a plastic adhesive sheet (SealPlate®; EXCEL Scientific, Inc., CA) was placed over each plate to prevent volatiles in the clove oil from escaping to other wells. Numbers of viable J2 and of hatched eggs were counted after incubation at 28 °C for 48 h and 7 days respectively. To determine if effects on J2 were reversible, the treatments were removed in four assay trials, sterile distilled water was added to the wells containing the J2 and, 24 h after adding the water rinse, the numbers of mobile and immobile J2 were counted.

Six assays were conducted to determine the effect of clove oil on *M. incognita* egg hatch, and seven assays

were conducted to determine the effect on J2. On two occasions, two simultaneous trials with the same egg populations were conducted with different bottles of clove oil. Tested concentrations in four of the egg assays and four of the J2 assays were: 0.0% clove oil (water control), 0.0% clove oil (carrier control) and 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0% clove oil (volume clove oil/volume carrier solution). Two of these were simultaneous assays. The other two simultaneous assays for eggs and for J2 did not include 0.6% and higher concentrations. One J2 assay was conducted with concentrations of 0.0% clove oil (water control), 0.0% clove oil (carrier control) and 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26, 0.28 and 0.30% clove oil.

For assays of fumigant toxicity to nematodes, eggs and J2 were placed in wells containing water or carrier, and only one concentration of clove oil – 0.2, 0.5 or 5.0% – was applied to each plate. Fifteen wells, located closely together, were used on each plate. Five wells in a row contained clove oil formulation only, five wells in an adjacent row contained carrier with nematodes and five wells in the other adjacent row contained water with nematodes, so that there were five replicates per treatment per trial. Controls were nematodes in carrier or in water that were not exposed to volatiles from clove oil, with five or ten replicate wells per trial. The total number of wells $n = 5$ –25 per treatment. Each well received 1 mL of a clove oil concentration or 0.1 mL nematode inoculum (eggs or J2) in 0.9 mL sterile distilled water or carrier. The trials were conducted with ca 100 eggs and either 25 or 50 J2 in each well. The hard plastic lids that were provided with the microwell plates were used rather than the adhesive plastic sheets to allow for movement of volatiles from the clove oil to the wells containing nematodes; controls were in separate plates with empty wells nearby or were covered by adhesive sheets. Percentages of viable J2 were determined after 48 h incubation at 28 °C. In egg assays, percentages of hatched eggs were determined after incubation at 28 °C for 1 week. In the same assays, the viability of J2 that had hatched from the ca 100 eggs/well was also determined after 1 week.

2.3 Soil tests of clove oil activity against *Meloidogyne incognita*

Second-stage juveniles were prepared as described above. Freezer bags measuring 17.7 \times 20.3 cm (946 mL capacity) were each filled with 50 g of a sandy soil mix (16 sand:9 compost) that had been steamed and air dried. Each bag received 1000 J2 in 0.5 mL water and 4.5 mL of one clove oil concentration, and the bags were then closed. In two of the four trials, tested clove oil concentrations were 0.1, 0.15, 0.2, 0.25, 0.3 and 1.0% (volume clove oil/volume carrier solution), 0% (water) and 0% (carrier). In the other two trials, the tested concentrations were 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0%, 0% (water) and 0% (carrier). Seven days later the soil was placed onto Baermann funnels

lined with double layers of tissue, and the J2 were collected after 48 h.³⁰ Total numbers of J2 recovered from each funnel were counted. Five replicate funnels were used per treatment per trial.

2.4 Gas-liquid chromatography of clove oil

Percentages of major constituents of clove oil were not listed on the bottles used for these experiments, and the chemical composition of clove oil can vary with geographic origin or source plant part. To determine whether any gross variations among major constituents existed between different lots of clove oil or among bottles of the same lot, several bottles of clove oil were selected for testing to ensure that any variability that might occur among trials was not caused by variability in amounts of the major constituents. The following four bottles were tested: Sigma C8392-100 mL, batch 125K1383, unused bottle; Sigma C8392-100 mL, batch 83H0871, used for microwell assays; Sigma C8392-100 mL, batch 035K1403, used for microwell assays; Sigma C8392-100 mL, batch 125K1383, used for a soil trial. Each bottle was sampled 2-9 times. A Varian Model 3700 instrument (Varian, Inc., CA) equipped with a flame ionization detector and J&W fused silica capillary column (Agilent J&W, CA), 15 m × 0.32 mm ID, 0.25 μm film of DB-1, was used for gas chromatography. The temperature program was 40 °C for 5 min, with an increase of 5 °C per min to 240 °C. Chromatographic standards included eugenol, eugenyl acetate, β-caryophyllene, caryophyllene oxide and α-humulene (Sigma). These standards were selected on the basis of clove oil analyses in the scientific literature.^{7,12,15,22,31}

2.5 Statistical analyses

The percentages of hatched eggs and viable J2 in the microwell assays in which nematodes were immersed in clove oil were each calculated using Abbott's formula³² to adjust for unhatched eggs and J2 inactivity, respectively, in the carrier control. For each application of Abbott's formula,³² when the response percentage observed at a specific clove oil concentration was less than the percentage unhatched or non-viable in the control, the resulting negative estimate was set to zero. The relationship between concentration and percentage hatched eggs or percentage active J2 was modeled for each independent trial by fitting a log-logistic regression model³³ (percentage hatched eggs or percentage active J2 = $D + (A - D) / (1 + \exp[B * \log_e(\text{percentage concentration} / EC_{50})])$) (Proc NLIN; SAS, Cary, NC) to the five observed percentage hatched or percentage active values (i.e. wells) for each of the observed concentrations. The parameters A and D represent the upper and lower asymptotes of the curve. The parameter B indicates the rate of change relative to percentage concentration. A single representation of the log-logistic relationship between percentage hatched eggs or viable J2 (see Figs 1A and B) was obtained by averaging the parameter estimates from the individual trial models; estimates of EC_{50}

and EC_{90} (effective concentration that caused a 90% reduction in egg hatch or J2 viability) and their 95% confidence intervals were included to summarize the observed among-trials variation.

The presence of a volatility effect or a carrier effect was assessed by conducting a two-way ANOVA (Proc MIXED; SAS, Cary, NC).^{34,35} Means were separated using Tukey's adjustment for multiple comparisons ($P < 0.01$). To analyze results of tests on clove oil nematotoxicity to J2 in soil, a separate log-logistic model was fitted to the data observed for each of four independent trials, using five funnels per concentration. A single representation of the log-logistic relationship between percentage J2 recovery (see Fig. 2) and clove oil formulation concentration was obtained by averaging the parameter estimates from the individual trial models; estimates of EC_{50} and EC_{90} and their 95% confidence intervals were included to summarize the observed among-trials variation. Water control and carrier control were compared among the four trials using a two-way ANOVA. All data were analyzed using SAS release 9.1.3.³⁶

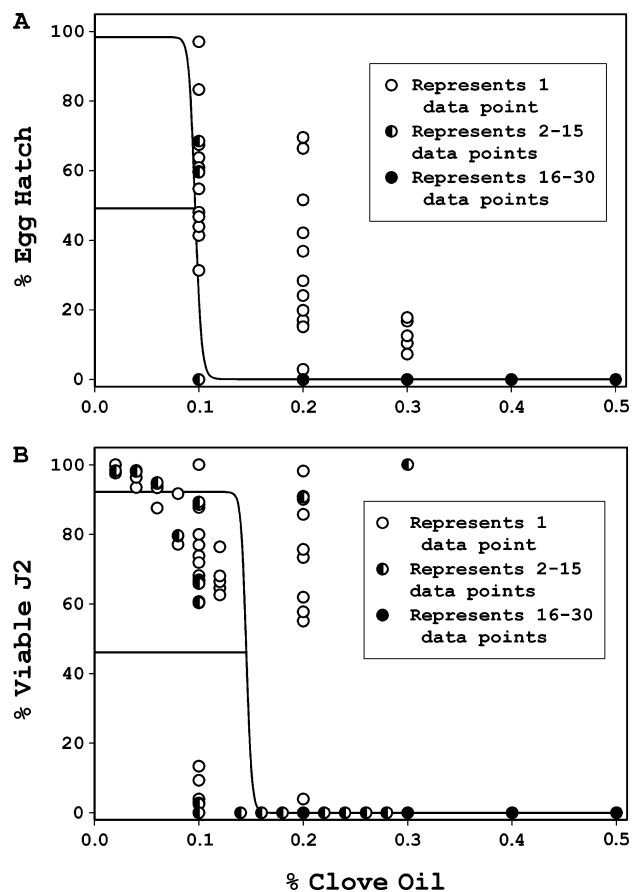


Figure 1. Dose-response curves for percentage *Meloidogyne incognita* egg hatch (A) and viable second-stage juveniles (J2) (B) in microwell assays following incubation in various concentrations of clove oil. Horizontal lines indicate 50% reduction in percentage egg hatch or in J2 viability. Percentages from microwell assays are based on numbers recovered from carrier control treatments and are adjusted for percentage unhatched eggs or percentage non-viable J2 in the carrier control, using Abbott's formula.³² Each curve represents the average across all trials, $P < 0.0001$.

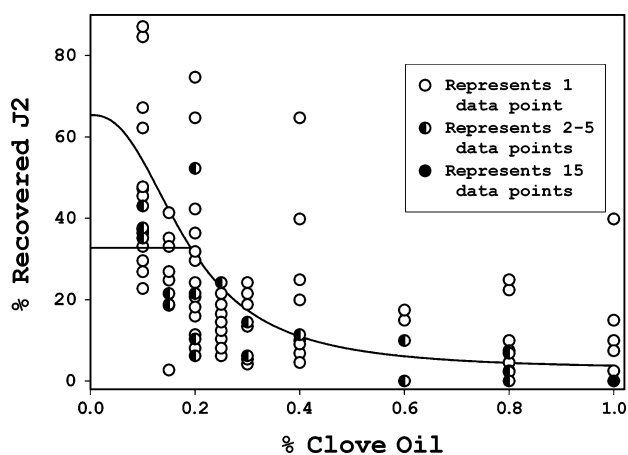


Figure 2. Dose–response curves for percentage *Meloidogyne incognita* second-stage juveniles (J2) recovered from soil treated with clove oil in Baermann funnel assays. The horizontal line indicates 50% reduction in J2 recovery. Percentages are based on J2 numbers recovered from water control treatments. The curve represents the average across all trials, $P < 0.0001$.

3 RESULTS

3.1 Microwell assays of clove oil activity against *Meloidogyne incognita*

Egg hatch responses to immersion in various concentrations of clove oil are presented with the carrier control as the ‘0% clove oil’ (Fig. 1A) (percentage eggs hatched = $0.108 + (98.36 - 0.108) / \{1 + \exp[32.84 * \log_e(\text{percentage concentration} / 0.096)]\}$) with rate of change in percentage eggs hatched per unit change in percentage clove oil concentration a function of 32.84 with standard error 18.6). Responses to clove oil varied among trials, but a regression model of all trials combined indicated that, overall, percentage egg hatch dropped with egg immersion in a concentration of approximately 0.1% clove oil (Fig. 1A). Consequently, the EC_{50} and the EC_{90} for egg hatch were very close together; $EC_{50} = 0.097\%$ clove oil (95% confidence intervals (CI_{95}) = 0%, 0.257%) and $EC_{90} = 0.104\%$ clove oil

($CI_{95} = 0\%, 0.383\%$). No carrier effect was observed for percentage eggs hatched.

The regression model of all trials with J2 immersed in clove oil is shown in Fig. 1B (percentage viable J2 = $92.17 / \{1 + \exp[58.06 * \log_e(\text{percentage concentration} / 0.145)]\}$) with rate of change in percentage viable J2 per unit change in percentage clove oil concentration a function of 58.06 with standard error 24.3). The dose–response curve indicates that J2 viability was reduced at approximately 0.14–0.15% clove oil (Fig. 1B). The clove oil EC_{50} and EC_{90} values for J2 viability were only slightly higher than those calculated for egg hatch. For J2 viability, the $EC_{50} = 0.145\%$ clove oil and the $EC_{90} = 0.151\%$ clove oil ($CI_{95} = 0\%, 0.456\%$ and $0\%, 0.465\%$ respectively). Unlike the egg assays, J2 were significantly ($P \leq 0.02$) more active in carrier controls than in the water controls for five of the seven trials, and when all trials were combined for comparison.

In several assays with J2 and clove oil, the clove oil treatments were removed and replaced with sterile water rinse for 24 h. Once a concentration was reached where 100% of the J2 were inactive, effects were irreversible, although some J2 did become active again at concentrations where less than 100% of J2 were inactive.

Volatiles from clove oil wells reduced percentage egg hatch (Table 1). When eggs were placed in carrier near 0.2, 0.5 and 5.0% clove oil, significant decreases in egg hatch were observed near the 5% clove oil concentration. Egg hatch in carrier near 5.0% clove oil was decreased by 18% compared with hatch in carrier controls not exposed to clove oil. When eggs were placed in water, there was a decrease in egg hatch near the 0.5 and 5.0% clove oil concentrations, with hatch decreased by 13 and 30%, respectively, compared with egg hatch in water controls. The carrier provided some protective effect for eggs against the volatiles from the nearby clove oil. Near concentrations of 0.5 and 5.0%

Table 1. Percentages (and standard errors) of *Meloidogyne incognita* egg hatch and of viable second-stage juveniles (J2) in water or in soy/lecithin carrier exposed to volatiles from clove oil in nearby wells on 24-well cell culture plates. Egg hatch was counted after 1 week exposure to volatiles, and viability of J2 that hatched from those eggs was counted at the same time. Viability of J2 hatched from eggs prior to use in the assays was counted after 48 h exposure

Nematode life stage (assay duration)	Percentage egg hatch or J2 viability ^a			
	Clove oil concentration			
	No clove oil ^b	0.2%	0.5%	5.0%
Eggs in carrier (1 week)	100 aA (2.1)	107 aA (4.1)	100 aA (2.4)	82 bA (3.1)
Eggs in water (1 week)	100 aA (2.4)	103 aA (3.8)	87 bB (3.9)	70 cB (2.4)
J2 hatched from assay eggs in carrier (1 week)	75 aA (3.8)	67 aA (4.1)	63 aA (2.8)	22 bA (1.6)
J2 hatched from assay eggs in water (1 week)	75 aA (4.4)	59 aA (2.8)	37 bB (7.0)	0 cB (0.1)
Hatched J2 in carrier (48 h)	97 aA (0.5)	95 bA (1.2)	91 bA (2.2)	86 cA (2.3)
Hatched J2 in water (48 h)	84 bB (1.7)	93 abA (0.9)	88 abA (1.7)	78 cB (3.5)

^a Lower-case letters are comparable only within rows. Upper case letters are only comparable for ‘eggs’ within a column, for ‘J2 hatched from assay eggs’ within a column and for ‘hatched J2’ within a column. For each life stage, main-effect means were separated using Tukey’s adjustment for multiple comparisons ($P < 0.001$).

^b Eggs and J2 were in carrier or in water and were not exposed to volatiles from clove oil.

clove oil, egg hatch in water was significantly less than hatch in carrier.

Volatiles from clove oil also decreased J2 viability. In the 1 week assays, some of the J2 were exposed to the volatiles for more than 48 h after hatching, and J2 viability in carrier was reduced by 70.7% near the 5.0% clove oil. Viability of J2 in water during the 1 week assays was significantly decreased near the 0.5 and 5.0% clove oil concentrations, with 50.7 and 100% reductions respectively. There was therefore a greater decrease in J2 viability than in egg hatch over the 1 week assay period (Table 1). There was also a stronger effect on J2 in the longer assays than in the assays with 48 h exposure of J2 to volatiles from clove oil. In the 48 h assays, viability of hatched J2 in carrier and in water was substantially reduced only near the 5.0% clove oil concentration (Table 1). Percentage viable J2 in carrier near 5.0% clove oil was decreased by 11.3% compared with controls, and percentage viable J2 in water near 5.0% clove oil was decreased only by 16.1% compared with the highest percentage activity (Table 1). However, there were common trends among all of the volatility assays. A protective effect of carrier was observed for eggs and J2. Also, in general, the volatiles from 0.2% clove oil (a concentration close to the EC₅₀ for J2 in soil recovery experiments) did not substantially affect eggs or J2.

3.2 Soil tests of clove oil activity against

Meloidogyne incognita

Percentage J2 recovered from carrier treatments was significantly ($P = 0.0004$) decreased by 34.2% compared with percentage recovery from water treatments. Because of this strong effect of the carrier in the soil experiments, the dose–response curve is presented with the water controls as the ‘0% clove oil’ treatment, so that effects of the entire formulation against nematodes in soil could be assessed (Fig. 2) (percentage recovered J2 = $2.82 + (65.38 - 2.82) / \{1 + \exp[2.46 * \log_e(\text{percentage concentration}/0.185)]\}$ with rate of change in percentage recovered J2 per unit change in percentage clove oil concentration a function of 2.46 with standard error 0.36). When trials were combined, the EC₅₀ = 0.192% and the EC₉₀ = 0.568% clove oil (CI₉₅ = 0%, 0.691% and 0.405%, 0.731% respectively). The EC₅₀ was therefore similar to that calculated for J2 in the microwell assays, but the EC₉₀ was higher. The highest tested concentration of clove oil, 1.0%, resulted in no recovery of J2 in three of the four trials. In the fourth trial, mean J2 recovery from 1.0% clove oil was 14.9% compared with water controls.

3.3 Gas–liquid chromatography of clove oil

Gas chromatographic analyses of the four analyzed bottles of clove oil yielded 14 consistently detectable components. The five most abundant components were identified on the basis of their identical retention times with standards known to be major components of clove oil. Amounts of the five components were

similar among the bottles and over sampling times, although different batches of clove oil had minor but consistently observable quantitative differences. The percentages of the five major components in the analyzed clove oils ranged from 74.6 to 78.6% (eugenol), from 9.5 to 11.3% (β -caryophyllene), from 10.3 to 11.7% (eugenyl acetate), from 1.01 to 1.42% (α -humulene) and from 0.38 to 0.55% (caryophyllene oxide).

4 DISCUSSION

In microwell assays, clove oil reduced *M. incognita* egg hatch and J2 viability. To a lesser extent, volatiles from wells containing clove oil were also toxic to the nematodes in nearby wells. However, this treatment was more effective in reducing viability of hatched J2 than in decreasing egg hatch, and was not as nematotoxic to either life stage as immersion in the clove oil formulation. The tested clove oil formulation was also active against *M. incognita* J2 in a soil environment, with a mean EC₅₀ value comparable with that recorded for J2 in the microwell assays.

Clove oil, being hydrophobic, needs to be formulated into an emulsion with surfactants for dilution with water. The carrier used to formulate the clove oil appeared to have a positive effect on the J2; there was some tendency for J2 to be more active in the carrier control than in the water control. Also, at the higher concentrations in the volatility studies, more eggs hatched in carrier than in water, and more hatched J2 were viable in carrier than in water. However, in the soil experiments, treatment with the carrier control resulted in a lower number of recovered J2 than treatment with the water control. The reasons for this difference between the microwell assays and the soil tests are unclear, but use of a carrier that tends to reduce nematode numbers without addition of clove oil could supplement nematode management efforts.

Previous studies have investigated effects of clove oil on nematodes, mostly on taxa other than *M. incognita*. Clove oil was irreversibly nematotoxic to J2 of *Anguina tritici* (Steinbuch) Chitwood, *Tylenchulus semipenetrans* Cobb, *Meloidogyne javanica* (Treub) Chitwood and *Heterodera cajani* Koshy.²⁶ The concentration of clove oil that resulted in 50% mortality (LC₅₀) of J2 populations of *M. javanica* (a species in the same genus as *M. incognita*) was 413 $\mu\text{g mL}^{-1}$, while the highest LD₅₀ value was 1040 $\mu\text{g mL}^{-1}$ for *H. cajani*. Extracts from clove also caused mortality in *Xiphinema americanum* Cobb, *Longidorus* sp. Micoletzky, *Hoplolaimus indicus* Sher, *Pratylenchus* sp. Filipjev and *Helicotylenchus indicus* Siddiqi when nematodes were exposed to 50, 75 and 100% concentrations.¹³ Nematotoxicity was observed when *Bursaphelenchus xylophilus* (Steiner & Buhner) Nickle J2 and adult nematodes were placed into wells with various concentrations of clove oil in distilled water + Triton X-100.²³ Extracts from flower buds were active against *B. xylophilus* at 1000 $\mu\text{L L}^{-1}$, causing

100% immobility to males, females and juveniles, but 500 $\mu\text{L L}^{-1}$ was not effective.²³ Aqueous extracts from *S. aromaticum* killed 50% of *Meloidogyne exigua* Goeldi J2 and inhibited egg hatch.²⁴ In a study with *M. incognita*, the nematode used in the present research, extracts from *S. aromaticum* flowers applied to bean plants (*Phaseolus vulgaris* L.) reduced nematode populations, although *S. aromaticum* extracts increased *M. exigua* numbers on coffee (*Coffea arabica* L.).²⁵ Clove oil extract did not reduce *Aphelenchoides fragariae* (Ritzema Bos) Christie on hosta (*Hosta* spp. Tratt.).³⁷ As indicated by these studies, clove oil has shown variable results when used for nematode management. Only one of these studies examined effects on *M. incognita*, and, as in the present study, nematode populations were reduced in the soil.

The main clove oil constituent, eugenol, has also been tested against nematodes. Eugenol was nematotoxic to *A. tritici*, *Caenorhabditis elegans* (Maupas) Dougherty, *H. cajani*, *M. javanica*, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven and *T. semipenetrans*.^{1,26,38} The LD₅₀ values reported with clove oil were higher than the values recorded with eugenol for *A. tritici*, *T. semipenetrans* and *H. cajani*, but lower for *M. javanica* in clove oil than in eugenol.²⁶ Eugenol was nematotoxic to *M. incognita* at 660 $\mu\text{g mL}^{-1}$,³⁹ and it reduced mobility of *M. incognita* and *H. cajani* at 500 ppm, with less activity at lower tested concentrations.⁴⁰ A commercial standard of eugenol was also toxic to *M. incognita* J2.⁴¹ Eugenol at 50, 250 and 500 mg oil kg^{-1} soil did not affect *M. arenaria* or *M. incognita* galling of tomato (*Lycopersicon esculentum* Mill.), although there was a trend towards reduction in *M. arenaria* galling with 1500 mg kg^{-1} .⁴²

Although eugenol is the primary constituent of clove oil, other compounds are present, and the use of clove oil, rather than eugenol alone, allows for combined activity of nematicidal compounds. Clove leaf oil can have more than 30 constituents, including α -humulene (shown to be repellent to the pine wood nematode *B. xylophilus*⁴³). Examples of amounts of major clove oil constituents include 70.0–82.6% eugenol, 7.2–19.5% β -caryophyllene, 1.2–12.1% eugenyl acetate, 0.8–2.1% α -humulene and 0.3–0.4% caryophyllene oxide (ranges are combined data from clove leaf essential oil, analysis of clove oil from Sigma and two bud-derived oil samples).^{12,15,31} We detected at least 14 components in the clove bud oil purchased from Sigma, including the five major constituents recorded in the results. The numbers of compounds in clove oil and the concentrations of these compounds are variable, with factors such as geographic source and plant part playing a role.^{7,15} It is likely that some variability in action of clove oil against nematodes is a result of differences in clove oil compositions.

In summary, the present results demonstrated that the tested formulation, which contained bud-derived clove oil in a soy lecithin/detergent formulation, was active against *M. incognita* eggs and J2 in microwell assays and against J2 in soil tests. Volatiles from

clove oil were also nematotoxic, but it took a higher concentration of clove oil adversely to affect nematodes through volatility than was needed for immersed nematodes. Consequently, optimal use of clove oil as a management agent would likely occur with nematodes in direct contact with the formulation. In addition, the percentages of the main constituents in the tested clove oil were also determined, so that the results are now associated with known percentages of major clove oil components. Few studies have been conducted with clove oil and *M. incognita*, and the present results aid in determining how clove oil can be used to reduce populations of this nematode.

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