Sensitivity of *Meloidogyne javanica* and *Tylenchulus semipeneterans* to Isothiocyanates in Laboratory Assays

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**ABSTRACT**


Isothiocyanates are released through enzymatic degradation of glucosinolates produced by plants in the family Brassicaceae. Glucosinolate profiles differ among plant species and the isothiocyanate derivatives differ in their toxicity to nematodes. Control of plant-parasitic nematodes in soil by isothiocyanates released from incorporated brassicaceous plant material has been inconsistent. Success might be improved with knowledge of the relative toxicities of various isothiocyanates against nematodes. Laboratory assays were conducted to determine lethal concentration (LC) values in sand of seven commercially available isothiocyanates against *Tylenchulus semipeneterans* and *Meloidogyne javanica*. The LC$_{90}$ values were 0.01 and 0.03 µmol/ml for 2-phenylethyl isothiocyanate and 0.48 and 0.35 µmol/ml for phenyl isothiocyanate for *T. semipeneterans* and *M. javanica*, respectively. Brassicaceous sources of benzyl or 2-phenylethyl isothiocyanate and, to a lesser extent allyl isothiocyanate, are the most promising candidates for plant-parasitic nematode management. The broader context of this research is the development of approaches for consistent and reliable use of plant-derived chemicals for nematode management. The strategy is to select plants in the family Brassicaceae based on their glucosinolate profiles and the sensitivity of the target nematode species to the associated isothiocyanates.

Additional keywords: *Brassica hirta*, *B. juncea*, *B. napus*.

Plants in the family Brassicaceae produce glucosinolates that are thought to function as a defense against insect attack (5,11,23). Glucosinolates are β-D-thioglucosides distinguished from one another by differences in their organic side chains (R groups). Based on these differences, glucosinolates are grouped as either aliphatic, aromatic, or indole forms. They occur in all plant tissues and degrade via enzymatic hydrolysis (1). As a result of tissue damage, the relatively nonreactive glucosinolates come into contact with myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1), which is stored separately in the cell. Hydrolysis yields nitriles, epithionitriles, thiocyanates, and/or isothiocyanates depending on the parent glucosinolate, pH, and other factors (Fig. 1). Isothiocyanates are highly toxic compounds of varying volatility (8,23). They are general biocides whose activity results from irreversible interactions with proteins (5,24).

There is evidence that glucosinolate degradation products are toxic to nematodes. Nematodes were exposed to glucosinolates purified from brassicaceous tissue in the presence or absence of the enzyme myrosinase. With myrosinase present, glucosinolate degradation products were toxic, whereas in the absence of the enzyme intact, glucosinolates were not toxic (7,9,13,15,17).

The products of glucosinolate hydrolysis, assumed to be isothiocyanates, caused differing nematode mortality rates dependent on plant species, cultivar and growth stage (13), chemical composition of the side chain, concentration, and exposure time (17). The isothiocyanate products of the glucosinolates sinigrin, glucoraphin, leucotropolin, and glucocrociacin were nematicidal to *Heterodera schachtii* Schmidt, whereas those from glucoraphin and sinalbin were not (17). Allyl, benzyl, and 2-phenylethyl isothiocyanates were 100% lethal to *Globodera rostochiensis* Wollenweber, but 3-butenyl and 4-methylsulfanyl(butyl) isothiocyanates were less effective (7).

An extensive knowledge base exists regarding glucosinolate-producing brassicaceae species, their enzymatic isothiocyanate products, and their ability to suppress soilborne pathogens (5,8). However, laboratory successes have not always resulted in reliable plant-parasitic nematode management systems in soil. Factors to be considered in extending these studies to practical management systems include, but are not limited to, plant genus and species, plant age, and glucosinolate profile; target nematode species and life stage; and soil properties (5).

Numerous laboratory assays have been conducted to evaluate specific glucosinolates and isothiocyanates for plant-parasitic nematode suppression. Clearly, optimization of brassicaceae-based management systems will require a multilevel experimental approach.

This study represents the first part of a step-wise process that includes laboratory and field experiments. With the knowledge that the toxicity of isothiocyanates is dependent on the structure of the compound and the nematode species being targeted, the objectives of this study were to (i) determine the effect of different concentrations and types of commercially available isothiocyanates against plant-parasitic nematodes and (ii) quantify lethal concentration (LC$_{50}$ and LC$_{90}$) values of those isothiocyanates that have potential for application as brassicaceous plant material for nematode suppression in soil.

**MATERIALS AND METHODS**

Collection of assay nematodes. Mixed stages of *Tylenchulus semipeneterans* Cobb were extracted by decanting and sieving from soil from an infested olive orchard in Orland, CA. Extracted nematodes were placed on a Baermann funnel and nematodes were collected after 24 h and used immediately (14). Two-day-old second-stage juvenile *Meloidogyne javanica* (Treub) Chitwood were obtained from hydroponic tomato cultures (16) and used immediately.

**Assay chemicals.** Allyl, benzyl, butyl, ethyl, phenyl, 2-phenylethyl (Sigma-Aldrich Chemical, St. Louis), and 4-methyl-
sulfinyl(butyl) (IKT Technologies, Madison, WI) isothiocyanates were tested (Table 1). Purity of all isothiocyanates was at least 97%. Metham sodium (AMVAC, Los Angeles, CA), a methyl isothiocyanate liberator, was also tested. The formulation of metham sodium was 42% sodium methylthiocarbamate (anhydrous) and 58% inert ingredients. A dilution series of each chemical was prepared in methanol (24) so that the required amount of isothiocyanate was delivered in 50 µl. Stock solutions were stored in glass containers at –20°C.

**Assay protocol.** Autoclaved silica sand (3.1 cm³; No. 60 silica sand, Corona Industrial Sand, Corona, CA) was weighed into 3- × 2.7-cm polyvinyl chloride (PVC) tubes sealed at one end with a rubber stopper. Approximately 250 to 300 assay nematodes were placed on a soil volume basis.

<table>
<thead>
<tr>
<th>Isothiocyanate</th>
<th>Plant species</th>
<th>Plant part</th>
<th>Structure of side chain R</th>
<th>Molecular weight</th>
<th>Glucosinolate common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allyl</td>
<td>Armoracia lapathifolia, Brassica juncea, B. napus, B. oleracea</td>
<td>Seed, leaf, root, stem</td>
<td>CH₂(CH₃)₂CH₃</td>
<td>99.2</td>
<td>Sinigrin</td>
</tr>
<tr>
<td>Benzyl</td>
<td>Carica papaya, B. hirta, Lepidium sativum</td>
<td>Seed, leaf, root, stem</td>
<td>CH₃(CH₂)₂CH₂</td>
<td>149.2</td>
<td>Glucotropeolin</td>
</tr>
<tr>
<td>Butyl</td>
<td>A. lapathifolia, Capparis flexuosa</td>
<td>Seed, leaf, root, stem</td>
<td>CH₂(CH₂)₂CH₂</td>
<td>115.2</td>
<td>Glucolepdiin</td>
</tr>
<tr>
<td>Ethyl</td>
<td>Lepidium menziesi</td>
<td>Seed</td>
<td>CH₃(CH₂)₂CH₂</td>
<td>87.1</td>
<td>Glucocapparin</td>
</tr>
<tr>
<td>Methyl</td>
<td>Capparis spp.</td>
<td>Seed</td>
<td>CH₃(CH₂)₂CH₂</td>
<td>73.1</td>
<td></td>
</tr>
<tr>
<td>Phenyl</td>
<td>A. lapathifolia</td>
<td></td>
<td>CH₂(CH₂)₂CH₂</td>
<td>135.2</td>
<td></td>
</tr>
<tr>
<td>4-Methylsulfinyl(butyl)</td>
<td>B. oleracea</td>
<td>Seed, leaf, root, stem</td>
<td>CH₂(CH₂)₂S-CH₃</td>
<td>177.3</td>
<td>Glucoraphanin</td>
</tr>
<tr>
<td>2-Phenylethyl</td>
<td>A. lapathifolia, B. juncea, B. napus, B. hirta</td>
<td>Seed, leaf, root, stem</td>
<td>CH₂(CH₂)₂S-CH₃</td>
<td>163.2</td>
<td>Gluconasturtiin</td>
</tr>
</tbody>
</table>

*Data from Fahey et al. (11) and Brown and Morra (5).*

RESULTS

There were no differences in nematode survival between the water and methanol controls (data not shown). LC₅₀ and LC₉₀ values were determined for seven isothiocyanates and metham sodium (Table 2). Phenyl isothiocyanate was the least toxic to both nematodes followed by ethyl and butyl isothiocyanates. 2-Phenylethyl isothiocyanate was the most toxic to both nematodes.

Relative toxicities against *T. semipenetrans* for the isothiocyanates tested (in ascending order) were phenyl < (ethyl = butyl) < (metham sodium = 4-methylsulfinyl(butyl) = allyl) < (2-phenylethyl = benzyl). Although the order of toxicity changed for the LC₅₀ and LC₉₀ values, the isothiocyanates always fell into the same distinct toxicity groups.

The isothiocyanate LC₅₀ and LC₉₀ values for *M. javanica* were less consistent than those for *T. semipenetrans* (Table 2). At the LC₅₀ level, butyl, phenyl, and 4-methylsulfinyl(butyl) isothiocyanates were the least toxic to *M. javanica*, whereas 2-phenylethyl had the highest LC₅₀ value.
isothiocyanate was the most toxic. At the LC$_{50}$ level, butyl, 4-methylsulfinyl(butyl), phenyl, and ethyl isothiocyanates were the least toxic to *M. javanica*. The ascending order of relative toxicities of the most toxic isothiocyanates at the LC$_{50}$ level was metham sodium < benzy1 < 2-phenylethyl.

4-Methylsulfinyl(butyl) was the only isothiocyanate tested that differed greatly in its relative order of toxicity against the two nematodes. For *T. semipenetrans*, 4-methylsulfinyl(butyl) was always grouped in the second most toxic group with metham sodium. For *M. javanica*, 4-methylsulfinyl(butyl) was less toxic compared with most of the isothiocyanates tested (Table 2).

The LC$_{50}$ and LC$_{90}$ values for ethyl and phenyl isothiocyanates and for metham sodium were not different for the two nematodes. For allyl, benzy1, butyl, 2-phenylethyl, and 4-methylsulfinyl(butyl) isothiocyanates, the LC$_{50}$ and LC$_{90}$ values were always lower for *T. semipenetrans* than *M. javanica* (Table 2). The LC$_{50}$ values ranged from 2 times (butyl) to 15 times (benzy1 and 4-methylsulfinyl(butyl)) lower for *T. semipenetrans*. The LC$_{90}$ values of the tested isothiocyanates ranged from 1.75 times (butyl) to 10 times (4-methylsulfinyl(butyl)) lower for *T. semipenetrans* than for *M. javanica*.

The relative toxicity of individual isothiocyanates was different for each nematode. Benzyl, one of the most toxic isothiocyanates to *T. semipenetrans*, was 50 to 100 times more toxic than phenyl, the least toxic isothiocyanate tested. For *M. javanica*, the difference between the most toxic, 2-phenylethyl, and least toxic isothiocyanate, butyl, was only 10- to 15-fold.

Two examples of isothiocyanate response curves for the assay nematodes are presented in Figure 2. The response curve for phenyl isothiocyanate was not different for the two species. There was a difference in the response of *T. semipenetrans* and *M. javanica* to 2-phenylethyl isothiocyanate.

**DISCUSSION**

There was a wide range in toxicity among the isothiocyanates, with some 100 times more toxic than others. Relatively slight structural differences can confer profoundly different nematicidal effects, confirming that biological activity is a function not only of the concentration of the product but also of the chemical properties of the R side chain (17).

For both plant-parasitic nematodes tested, there was no clear relationship between structure or molecular weight of the isothiocyanates and their toxicities (Tables 1 and 2). Isothiocyanate toxicity is reported to increase with increasing volatility and decreasing molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Isothiocyanate toxicity is reported to increase with increasing volatility and decreasing molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower molecular weight (18,19).

Aliphatic isothiocyanates are expected to be more toxic to a range of organisms than aromatic isothiocyanates (3,18). In our study, aromatic isothiocyanates were the most toxic to both nematode species. Our results are similar to those of Sarwar et al. (24) who found that aromatic isothiocyanates were more toxic to soil-borne fungal pathogens than aliphatic isothiocyanates when dissolved in agar.

The use of commercially available isothiocyanates allows direct determination of LC$_{50}$ and LC$_{90}$ values by eliminating glucosinolate to isothiocyanate conversion and the possible presence of other alternative breakdown products (e.g., nitriles and epithionitriles) (Fig. 1). The types of assay systems used to determine isothiocyanate LC values vary greatly (9,20,24) but reveal general trends. Our LC$_{50}$ values for allyl isothiocyanate were several orders of magnitude lower than the LC$_{50}$ value reported in a solution assay for pure allyl isothiocyanate against *Caenorhabditis elegans* Dougherty and Nigon (9). The open system used by Donkin et al. (9) may have allowed greater volatilization of allyl isothiocyanate than in ours. Our LC$_{50}$ value for allyl isothiocyanate for *T. semipenetrans* was similar to the concentrations of allyl isothiocyanate lethal to five fungal pathogens (24). These concentrations were several orders of magnitude lower than our allyl isothiocyanate LC$_{50}$ value for *M. javanica*.

![Fig. 2. Response curves for percent reduction of *Meloidogyne javanica* (●) and *Tylenchulus semipenetrans* (◇) exposed to concentration ranges of A, 2-phenylethyl and B, phenyl isothiocyanates. Vertical bars represent the 95% confidence interval for each mean.](image-url)

**TABLE 2. Lethal concentrations values at 50 and 90% (LC$_{50}$ and LC$_{90}$) (µmol/ml) for *Tylenchulus semipenetrans* and *Meloidogyne javanica* for commercially available isothiocyanates**

<table>
<thead>
<tr>
<th>Isothiocyanate</th>
<th>T. semipenetrans</th>
<th>M. javanica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC$_{50}$</td>
<td>LC$_{90}$</td>
</tr>
<tr>
<td>Allyl</td>
<td>0.02 (0.02–0.03)</td>
<td>0.04 (0.03–0.05)</td>
</tr>
<tr>
<td>Benzy1</td>
<td>&gt;0.01 (&gt;0.01–∞)</td>
<td>&gt;0.01 (&gt;0.01–∞)</td>
</tr>
<tr>
<td>Butyl</td>
<td>0.11 (0.08–0.15)</td>
<td>0.27 (0.22–0.32)</td>
</tr>
<tr>
<td>Ethyl</td>
<td>0.14 (0.11–0.18)</td>
<td>0.23 (0.19–0.28)</td>
</tr>
<tr>
<td>Phenyl</td>
<td>0.25 (0.19–0.34)</td>
<td>0.48 (0.38–0.6)</td>
</tr>
<tr>
<td>2-Phenylethyl</td>
<td>&gt;0.01 (&gt;0.01–∞)</td>
<td>0.01 (0.01–0.01)</td>
</tr>
<tr>
<td>4-Methylsulfinyl(butyl)</td>
<td>0.02 (0.01–0.02)</td>
<td>0.05 (0.04–0.06)</td>
</tr>
<tr>
<td>Metham sodium</td>
<td>0.04 (0.02–0.07)</td>
<td>0.08 (0.05–0.11)</td>
</tr>
</tbody>
</table>

*All data was ln(1/(1 – y)) transformed and subjected to linear regression to obtain LC$_{50}$ and LC$_{90}$ values. Values in parentheses indicate 95% confidence intervals. Intervals overlapping within rows and columns are not significantly different.*
For 2-phenylethyl isothiocyanate, the reported LC_{50} value, using a different assay system, for *Pratylenchus neglectus* Rensch (20) was several orders of magnitude greater than our LC_{50} values for both nematodes. The UC mix (50/50 mix of peat and sand) used by Potter et al. (20) may have absorbed more 2-phenylethyl isothiocyanate because conversion rates were not determined.

There were no differences between the LC values for metham sodium against the nematodes. Metham sodium is a broad-spectrum fumigant (12) and might be expected to affect both species similarly. Metham sodium results are not directly comparable to material can then be applied based on isothiocyanate LC values.

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**LITERATURE CITED**


