Effect of fluensulfone on different functional genes of root-knot nematode *Meloidogyne incognita*

Alkesh Hada¹, Divya Singh¹, Kranti Kavalipurapu Veera Venkata Satyanarayana², Madhurima Chatterjee¹, Victor Phani³,* and Uma Rao¹,*

¹Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi, India.
²All India Coordinated Research Project on Nematodes, ICAR-Indian Agricultural Research Institute, New Delhi, India.
³Department of Agricultural Entomology, College of Agriculture, Uttar Banga Krishi Viswavidyalaya, Dakshin Dinajpur, West Bengal, India.

*E-mails: umarao@iari.res.in; victor@ubkv.ac.in

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Abstract

*Meloidogyne incognita* is an obligate plant-parasitic nematode causing serious damage to agricultural crops. Major constraints in nematode management arose due to the limited availability of non-fumigant nematicides in conjunction with the considerable ill effects of fumigants on human and non-target organisms. Recently, fluensulfone has been reported to be an effective non-fumigant nematicide against plant-parasitic nematodes and the model nematode *Caenorhabditis elegans*. The nematicidal efficacy varies according to its concentration at the time of application, exposure timing, nematode species variability, and even across subpopulations within the same species. It interferes with the key physiological processes of nematodes, like motility, behavior, chemosensation, stylet thrusting, infectivity, metabolism, lipid consumption, tissue integrity, oviposition, egg hatching, and survival. However, the molecular basis of these multivariate physiological anomalies is still largely unknown. Quantitative real-time PCR was carried out to understand the acute transcriptional perturbation of 30 functional genes associated with key physiological and life processes in a *M. incognita* population, following exposure of 10, 50, and 100 ppm of fluensulfone for 5 and 10 hr. The chemical treatment resulted in significant downregulation of all the neuropeptidergic genes, with concomitant repression of majority of genes related to chemosensation, esophageal gland secretion, parasitism, fatty acid metabolism, and G-protein coupled receptors. Collectively, the parasitism genes were found to be perturbed at highest magnitude, followed by the GPCRs and neuropeptidergic genes. These results establish the wide ranging effect of fluensulfone on various metabolic and physiological pathways of nematode.

Keywords

Chemosensation, Fluensulfone, Metabolism, Neurotransmission, Parasitism, Root-knot nematode.

Over 4,100 species of plant-parasitic nematodes (PPNs) pose a major threat to the present day agriculture accounting an estimated yield loss of US$ 173 billion every year (Decraemer and Hunt, 2006). Amongst the top 10 PPN species that cause majority of the economic damage worldwide, root-knot nematodes (RKNs) of genus *Meloidogyne* are considered to be the most severe (Elling, 2013; Jones et al., 2013). The second-stage juveniles (J2s) of RKNs enter the plant roots and develop permanent feeding sites (giant cells) that nurture them for rest of their growth and reproduction (Berg et al., 2009). While doing so, these microscopic animals develop a complex nexus of interactive cross-talks with their...
hosts and remarkably reprogram the plant cells for their own benefit. A wide array of nematode-derived effect or molecules, viz., the cell-wall modifying enzymes (Mantelin et al., 2017), esophageal gland secretions (Chaudhary et al., 2019; Ding et al., 2000; Huang et al., 2003); neurotransmitters (e.g., acetylcholine, FLPs, NLPs) (Blanchard et al., 2018; Dash et al., 2017; Kumar et al., 2017; Papolu et al., 2013), chemosensory genes (Fleming et al., 2017; Shivakumara et al., 2019), etc. play pivotal roles during this parasitism process to overcome the plant-foisted go/no-go checkpoints. All the key functional genes collectively contribute in shaping a compatible nematode-plant interaction, which ultimately affect the crop yield from agricultural viewpoint.

In spite of the enormous damage caused by the PPNs to agricultural crops, there still remains an acute scarcity of effective and efficacious nematode management option(s). Predominantly, the management of PPNs is traditionally relied on integrated cultural, physical and biological means with use of insecticidal chemicals (Bridge, 1996; Dutta et al., 2019). But, of late, many of the well-known ‘nematicides’ including the fumigants and insecticides are phased out for their undesirable effects on nature (Kim et al., 2017; US-EPA, 2008, 2009). Amongst the novel nematicides, fluensulfone, fluopyram, fluazaindolizine, and toxazafenate have been proven to be highly effective against the PPNs (Faske and Hurd, 2015; Kearn et al., 2014; Lahm et al., 2017; Slomczynska et al., 2014).

Considering these four chemicals, fluensulfone has shown excellent results in controlling the nematodes with unique mode of action (MoA), and is being widely used across the globe (Oka, 2014; Oka et al., 2008, 2009, 2012, 2013). Unlike the fumigants, fluensulfone has very low toxicity toward non-target organisms and it does not emit any volatile organic compound(s) (Ploeg et al., 2019; Waldo et al., 2019). Exposure to this chemical exerts irreversible nematicidal effects affecting the motility, chemosensory perception, stylet thrusting, feeding, moulting, infection potential, oviposition capacity, egg hatching, behaviour, metabolism, lipid consumption, tissue integrity and survival in root-knot, cyst and other nematode species (Kearn et al., 2014, 2017; Oka and Saroya, 2019; Wram and Zasada, 2019). Chemically, fluensulfone [5-chloro-2-(3,4,4-trifluorobut-3-enylsulfonyl)-1,3-thiazole] belongs to heterocyclic fluoroalkenylsulfone group and acts via contact toxicity on the nematodes when directly applied in soil (Kearn et al., 2014). Exposure of PPNs and Caenorhabditis elegans against this chemical resulted in non-recoverable paralysis of the worms with characteristic ‘rod-shaped’ body posture, unlike the resultant ‘wavy’ paralysis due to cholinesterase inhibitors (Oka et al., 2009). Behavioral and electrophysiological studies in C. elegans following acute and chronic exposure to fluensulfone revealed its effect to be distinct from the organophosphates, carbamates, and macrocyclic lactones (Kearn et al., 2014). The study also demonstrated that the embryo and larval stages of C. elegans are more susceptible toward this chemical, and it inhibits the nematode feeding, moulting, and egg hatching possibly by targeting the mitochondrial function (Kearn et al., 2014). Further investigation with plant-parasitic species Globodera pallida also revealed that fluensulfone progressively immobilizes the pre-parasitic J2s in a time and concentration dependent fashion, and compromises the internal integrity of the worm (Kearn et al., 2017).

All these reports describe the multifaceted effect of fluensulfone on nematode biology and physiology. However, the underlying gene regulation mechanisms causing such physiological anomalies have not been fully understood.

In the present study, we have tested the effect of fluensulfone on expression of 30 functional genes in an Indian subpopulation of the root-knot nematode M. incognita. For this purpose, representative genes associated with chemosensation, esophageal gland secretion, nematode parasitism, fatty acid metabolism, β-oxidation, polyunsaturated fatty acid (PUFA) fractionation, neurotransmission, and G-protein coupled receptors (GPCRs) were selected from M. incognita. The effects were observed by directly treating the nematode J2s with different concentrations of fluensulfone (10, 50, and 100 ppm), followed by analysis of transcript levels of the respective genes by quantitative real-time PCR (qRT PCR) at two time points (5 and 10 hr post exposure).

Materials and methods

Nematode population and drug material

The pure culture of an Indian isolate of Meloidogyne incognita race 1 was raised on susceptible tomato plants (Solanum lycopersicum cv. Pusa ruby) in a glasshouse at ICAR-Indian Agricultural Research Institute, New Delhi, India. The nematode infected roots were washed free of soil, eggmasses were hand-picked and hatched via ‘modified Baermann’s funnel technique’ (Whitehead and Hemming, 1965). The freshly (within 24 hr) hatched second-stage juveniles (J2s) were used for experimental purpose.

Fluensulfone was procured from Sigma-Aldrich (purity: 99.99%; Sigma-Aldrich, St. Louis, Missouri, USA) only for experimentation and was stored at 4°C
in presence of desiccant silica granules. The chemical was then dissolved in an organic vehicle, i.e., dimethyl sulfoxide (DMSO; pure grade) to prepare a carrier solution of 20,000 ppm (1 mg fluensulfone in 50 µL DMSO). The carrier solution was then dissolved in desired quantity of nuclease free water to achieve 10, 50, and 100 ppm of final fluensulfone concentration, to be used for soaking purpose.

Chemical exposure, RNA extraction, and cDNA preparation

The *M. incognita* J2s were soaked in three concentrations of fluensulfone (10, 50, and 100 ppm) for exposure, and the effect was studied at 5 and 10 hr post soaking. Soaking was continued in dark on a slowly moving rotator at room temperature (-28°C) maintaining three biological replicates; and J2s soaked in 0.05% DMSO (vehicle control) and M9 buffer (1 mM MgSO4, 22 mM KH2PO4, 42.3 mM Na2HPO4, and 85.6 mM NaCl; pH 7.0) for 5 and 10 hr served as controls. Around 5,000 J2s were used for each replication. Following soaking, total RNA was extracted from the J2s with NucleoSpin RNA kit (Macherey-Nagel, Düren, Germany) according to manufacturer’s protocol as described previously (Phani et al., 2018). The RNA was treated with RQ1 RNase-Free DNase (Promega, Madison, WI, USA) to get rid of any genomic DNA contamination. The integrity and quality of RNA was determined by 1% agarose gel and NanoDrop-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Approximately 500 ng of RNA was reverse transcribed into total cDNA using cDNA synthesis kit (Superscript VILO, Invitrogen, Carlsbad, CA, USA).

Analysis of mRNA levels by qRT PCR

A total of 30 genes were selected in the present study governing diverse physiological and behavioral activities in *M. incognita*, amongst which 12 genes (osm-9, gpc-1, gpa-11, gpa-13, grk-2, fat-6, acs-2, mdt-15, nhr-49, elo-2, ech-5, and ech-6) were identified and cloned from *M. incognita* for the first time (Table 1) (Ashrafi, 2007; Bargmann, 2006; Jansen et al., 2002; Lans and Jansen, 2007; Wang et al., 2017). For this, comparative genomics were used to putatively identify the *M. incognita* orthologs of the previously unannotated genes using *C. elegans* genomic database as query (Supplementary Table 1). The respective protein sequences (of *C. elegans* genes) were retrieved from WormBase (Version: WS260) database, and were BLAST searched in WormBase Parasite (http://parasite.wormbase.org/Tools/Blast) and INRA database (http://www6.inra.fr/meloidogyne_incognita/Genomic-resources2/Blast) to fetch the translated nucleotide sequences in *M. incognita*. Identity of the exact putative gene hits in *M. incognita* was deputed from the sequences having smallest expect value and large bit score. The sequences were then checked for presence of conserved domains and specific primers were designed to amplify the genes from *M. incognita* cDNA. The amplified products were then cloned, sequenced via Sanger sequencing for reconfirmation, and the sequences were submitted to NCBI GenBank (Table 1).

Quantitative real-time PCR (qRT PCR) was carried out to analyze the expression pattern of the genes in *M. incognita* J2s after exposing them against fluensulfone. The qRT PCR was performed in a Realplex2 thermal cycler (Eppendorf, Hamburg, Germany) using SYBR Green Supermix Kit (Eurogentec, Liege, Belgium) (Phani et al., 2018). Reaction mixture for each sample contained a final volume of 10 µL comprising of 5 µL of SYBR Green PCR Master mix, 750 nM of each primers and 1.5 ng of cDNA. Gene expression level was normalized using two constitutively expressed *M. incognita* genes, 18S rRNA (HE667742) and actin (BE225475). For each of the analyzed genes, three biological and three technical replicates were used, data were analyzed by 2^(-ΔΔCt) method (Livak and Schmittgen, 2001), and fold change expression values were subjected to Student’s t test for determining the statistical significance (p=0.05, 0.01).

Results

Gene amplification

A total of 12 genes (out of 30) were identified and cloned in the present study for qRT PCR purpose, which were previously unannotated in *M. incognita* (Supplementary Table 1). For this, reciprocal best hit approach was optimized at ≥30% identity, ≥50% query coverage, and <10^-5 E-value for identification of the gene orthologs. Thereafter in silico validation and analyses confirmed the presence of respective conserved motifs in the sequences; and were submitted to NCBI GenBank (Table 1). Owing to the experimental integrity for qRT PCR purpose, concentration of fluensulfone and time of exposure was combined in such way that no worm dies, and nematicidal effect was microscopically assessed by behavioral changes, immobility and mortality in the *M. incognita* J2s (Supplementary Table 2).
Table 1. List of primers used in this study.

| Sl | Primers name       | Gene name (accession number)       | Primers Sequences (5′–3′) | Tm (°C) | Purpose              |
|----|--------------------|------------------------------------|----------------------------|---------|----------------------|
| 1  | RT miODR-1 F       | Mi-odr-1 (MG780832)               | GAACCACGCTCTTTACGATTC      | 60°C    | qRT PCR              |
|    | RT miODR-1 R       |                                    | CTCCAAAAGCGACCATGTA        |         |                      |
| 2  | RT miODR-3 F       | Mi-odr-3 (MG780833)               | CTGGCTATAGACCACCAAGAA     | 60°C    | qRT PCR              |
|    | RT miODR-3 R       |                                    | GAACGTGTCACCTACTCATC      |         |                      |
| 3  | RT miTAX-2 F       | Mi-tax-2 (MG780834)               | GAGGGAAATATCTCAGAGG       | 60°C    | qRT PCR              |
|    | RT miTAX-2 R       |                                    | CCTGATCCACTGTTCTGG        |         |                      |
| 4  | RT miTAX-4 F       | Mi-tax-4 (MG780835)               | GTGAAGTTCTTGCCCTAT        | 60°C    | qRT PCR              |
|    | RT miTAX-4 R       |                                    | CAGAGCTGCAAATCTACTCTC     |         |                      |
| 5  | RT miOSM-9 F       | M. incognita osm-9 (MT676864)     | CATGCCTGAAGATGGGGAAG      | 60°C    | Cloning and qRT PCR |
|    | RT miOSM-9 R       |                                    | GGTCAGGATTAGCACCATCAC     |         |                      |
| 6  | RT miGPC-1 F       | M. incognita gpc-1 (MT676857)     | CAACCTCGACAAAGGGCAA       | 60°C    | Cloning and qRT PCR |
|    | RT miGPC-1 R       |                                    | CCCTATTTGCTCGGTAATTAAG    |         |                      |
| 7  | RT miGPA-11 F      | M. incognita gpa-11 (MT676854)    | CGACTACGGGTATTTGTGAC      | 60°C    | Cloning and qRT PCR |
|    | RT miGPA-11 R      |                                    | GAAAGTCGCTACGAAAGACAG     |         |                      |
| 8  | RT miGPA-13 F      | M. incognita gpa-13 (MT676855)    | GCTGAAACCTCGAGAGGAGG     | 60°C    | Cloning and qRT PCR |
|    | RT miGPA-13 R      |                                    | GCTGGTATTCACGAGAACG       |         |                      |
| 9  | RT miGRK-2 F       | M. incognita grk-2 (MT676858)     | CTCAATTTCGCAGAGGAAGAG     | 60°C    | Cloning and qRT PCR |
|    | RT miGRK-2 R       |                                    | GGTGCCATATTACGCAAAGAG     |         |                      |
| 10 | RT miXYL-1 F       | Mi-xyylanase (AF224342)            | GGGATTAGTGGCATACAGGGAAGAG | 60°C    | qRT PCR              |
|    | RT miXYL-1 R       |                                    | GCTGGTACGAGTACGAG         |         |                      |
| 11 | RT miXYL-3 F       | Mi-xyylanase (EU475876)            | GGAACGTTCGGGTAACACCTTA   | 60°C    | qRT PCR              |
|    | RT miXYL-3 R       |                                    | GACCGGACTGATGTGTACATC     |         |                      |
| 12 | RT miENG-1 F       | Mi-β-1,4-endoglucanase (AF100549) | ACCGAGCAAACCTCAAAC       | 60°C    | qRT PCR              |
|    | RT miENG-1 R       |                                    | GCCATTTGCCGCGATATT        |         |                      |
| 13 | RT miPEL F         | Mi-pectatelyase (AF527788)         | CAAATAAGCAAGAAGAGG       | 60°C    | qRT PCR              |
|    | RT miPEL R         |                                    | AGAACCGCAAACCTACA         |         |                      |
| RT miMSP-20 F | Mi-msp-20 (AY134439) | TGGTGACGAACGCACACCACACACACATA | 60°C | qRT PCR |
| RT miMSP-20 R | | GCGCTGCTTTTGACCATATTT | |
| RT miMSP-33 F | Mi-msp-33 (AY142118) | GTGGGCCTCTTTGCTTGGACATTT | 60°C | qRT PCR |
| RT miMSP-33 R | | CATCACCCAAATTACTCGGGTT | |
| RT miFAT-6 F | M. incognita fat-6 (MT676846) | GAATCTGACGAGTCCTCAAC | 60°C | Cloning and qRT PCR |
| RT miFAT-6 R | | CATCAGTGACGAGTCCTCAAC | |
| RT miACS-2 F | M. incognita acs-2 (MT676837) | CCCACCAGAGAAGGAAAT | 60°C | Cloning and qRT PCR |
| RT miACS-2 R | | CGCTCTATACCAACACATCT | |
| RT miMDT-15 F | M. incognita mdt-15 (MT676859) | CAGCTATGCCACCAGGTTTT | 60°C | Cloning and qRT PCR |
| RT miMDT-15 R | | CCTATTCCCTCCCCACACATTTTC | |
| RT miNHR-49 F | M. incognita nhr-49 (MT676861) | GCCAATAGGGCAATTAGAG | 60°C | Cloning and qRT PCR |
| RT miNHR-49 R | | CCTCAGCTACGGTTTATAG | |
| RT miELO-2 F | M. incognita elo-2 (MT676842) | CAAAACGGATATTGGGTCTGG | 60°C | Cloning and qRT PCR |
| RT miELO-2 R | | GGCCTGATGGGTAGGAATA | |
| RT miECH-5 F | M. incognita ech-5 (MT676839) | CACAGGGCTACGGACTGAA | 60°C | Cloning and qRT PCR |
| RT miECH-5 R | | CTTGGGAATCTTTGAGGTTCC | |
| RT miECH-6 F | M. incognita ech-6 (MT676840) | CGAGTCTTCTCAGAAGGATA | 60°C | Cloning and qRT PCR |
| RT miECH-6 R | | GCTTAGATGGGAAAGAGGTC | |
| RT miFLP-12 F | Mi-flp-12 (AY804187) | TGGAGAAAGGGCCCGATAGTTCTT | 60°C | qRT PCR |
| RT miFLP-12 R | | GAGATGAAAGAAGTGGGACGACT | |
| RT miFLP-14 F | Mi-flp-14 (AY907829) | GCGGATCGCTTGATGAGCT | 60°C | qRT PCR |
| RT miFLP-14 R | | GGGAGATGAAGGAAACGTGGTTTACTACGT | |
| RT miFLP-16 F | Mi-flp-16 (EU549831) | GCGCAATTTACGGAGAAGGAGA | 60°C | qRT PCR |
| RT miFLP-16 R | | GGCATTTACAGCTGTCAGGAGG | |
| RT miFLP-18 F | Mi-flp-18 (AY729022) | AGGATGACTTATTGCCAGGAGA | 60°C | qRT PCR |
| RT miFLP-18 R | | TTCCATTACGGAAATCTGGAGACAAG | |
| RT miACE-1 F | Mi-ace-1 (AF075718) | CTCCCTGTTCTAGAGGATTGTCT | 60°C | qRT PCR |
| RT miACE-1 R | | TTTATGGAGGCTTCTGAGGAG | |
| RT miACE-2 F | Mi-ace-2 (AF495588) | AGATGGAGATCCGGCTAATG | 60°C | qRT PCR |
| RT miACE-2 R | | TTTACTCGGGCTCTCCTTC | |
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**Effect on chemosensory and GPCR genes**

The DMSO (vehicle) control and M9 (buffer) control were insignificantly different, and the data representing comparative fold change expression between treatments and M9 buffer control were presented here. A total of nine chemosensory and GPCR genes (*odr-1, odr-3, tax-2, tax-4, osm-9, gpc-1, gpa-11, gpa-13, and grk-2*) were taken in the present study to check their expression level after the J2s being treated with different concentrations of the chemical. The exposure of J2s for 5 and 10 hr against all three fluensulfone concentrations resulted in downregulation of both the *odr* genes in a range of −0.08 to −3.08 folds (Fig. 1). In contrast, *tax-2* and *tax-4* were up- and downregulated against exposure to different time-concentration combinations (Fig. 1). *osm-9* was downregulated at both the time points against exposure of all the three concentrations. However, *gpc-1, gpa-11* and *gpa-13* and *grk-2* showed similar type of transcriptional perturbation with downregulation at 5 hr post exposure for all three concentrations; but upregulated at 10 hr against 50 and 100 ppm of fluensulfone.

**Effect on parasitism genes**

The parasitism genes studied here included the *M. incognita* effectors encoding cell wall modifying enzymes (CWMEs) and the esophageal gland secreted proteins. The genes encoding xylanase (*xyl-1* and *xyl-3*), β-1,4-endoglucanase (*eng-1*) and pectatelyase (*pel*) were downregulated at both the time points against exposure of 10, 50, and 100 ppm of fluensulfone (Fig. 1). In contrast, *msp-20* and *msp-33* showed downregulation only at 5 hr post exposure against 10 ppm of fluensulfone, but were upregulated at all other time-concentration combinations (Fig. 1). However, the transcriptional perturbation level of *xyl-1, xyl-3, eng-1* and *pel* was greater than *msp-20* and *msp-33*.

**Effect on fatty acid metabolism genes**

The fatty acid desaturase gene *fat-6* was downregulated at 5 hr post treatment against exposure to all the concentrations in a range of −1.23 to −1.72 folds; but was upregulated at 10 hr post treatment for all the concentrations to the tune of 0.16–0.99 folds (Fig. 1). The genes governing fatty acid elongation (*elo-2*) also showed similar type of transcriptional perturbation as of *fat-6* (Fig. 1). However, the genes involved in β-oxidation (*acs-2, mdt-15, nhr-49, ech-5*, and *ech-6*) processes were mostly downregulated.
after treatment at 10, 50, and 100 ppm for 5 and 10 hr, except slight upregulation was observed for ech-5 (0.80 fold) and ech-6 (0.78 fold) at 10 hr post exposure against 100 ppm of fluensulfone.

Effect on neuropeptidergic genes

Here, three types of neuropeptidergic genes representing acetylcholine, FMRFamide like peptides (FLPs) and neuropeptide like protein (NLPs) peptides were studied. Interestingly, all the genes were downregulated in the J2s at both the time points for all the fluensulfone concentrations. In the FLP category, flp-12, flp-14, flp-16, and flp-18 were downregulated in a range of −0.13 to −1.14 folds; along with the acetylcholinesterase genes ace-1 and ace-2 (Fig. 1). Similarly, expression of the NLP genes, nlp-3 and nlp-12 was repressed for all the time-concentration combinations in a range of −0.14 to −1.39 folds (Fig. 1).

Discussion

Here, we have determined the deviation of transcript abundance of 30 functional genes related to different physiological processes in M. incognita. The effect was determined after treating the nematode J2s with fluensulfone, an olefinic nematicidal compound of 1,3-thiazole class. The chemical exposure was achieved by direct soaking of the worms in 10 ppm (~34.28 µM), 50 ppm (~171.40 µM), and 100 ppm (~556.11 µM) of fluensulfone for 5 and 10 hr. Each bar represents the log2-transformed mean ± SE (n = 3). Asterisk (*) indicates significant differential expression (*p < 0.05; **p < 0.01; ns = non-significant), analyzed by one-tailed t-test.
ppm (~342.80 µM) of fluensulfone solution for 5 and 10 hr, and the toxicity effects were ascertained at transcriptional level by qRT PCR. Treatment of *M. incognita* J2s with fluensulfone largely brought a downregulated gene response of the tested genes; however, considerable variability can be observed with regard to this statement depending upon the gene classes and time-concentration combinations. Notably, all the neuropeptidergic genes were downregulated to variable extent for different time-concentration combinations. To the best of our knowledge, this is the first study deciphering the effect of fluensulfone on transcriptional perturbation of genes related to some vital physiological processes in any plant-parasitic nematode.

A considerable disparity lies in the concentrations of fluensulfone that bring nematcidal effect in the free-living species *C. elegans* and other plant-parasitic nematodes (Kearn, 2015; Oka, 2014; Oka et al., 2009). Oka et al. (2009) showed that fluensulfone exerts irreversible nematcidal activity in the *M. javanica* J2s with exposure of 12–48 hr against ≥3.4 µM concentration; and 3.4 µM fluensulfone resulted in chronic, non-spastic paralysis in the J2s with ≥80% mortality at 48 hr. The behavioral signs of nematcidal effect included uncoordinated body movement, reduced locomotion and pharyngeal pumping, defected host recognition, and characteristic body posture (Kearn, 2015; Oka and Saroya, 2019; Oka et al., 2009). However, the nematcidal effect of fluensulfone largely depends on concentration of the chemical, exposure timing, target nematode species, and even across population differences within the same species (Oka and Saroya, 2019; Shirley et al., 2019). In view of achieving the acute nematcidal response, three different concentrations (10, 50, and 100 ppm) of fluensulfone were used in the present investigation to expose the *M. incognita* J2s for relatively shorter time periods (5 and 10 hr). The time-concentration combinations kept the worms alive but exhibited deranged behaviour; and the effect was studied as transcriptional perturbation of the functional genes. The genes (studied here) were chosen for their involvement in diverse physiological processes and key life functions in *M. incognita*, such as chemosensation, neurotransmission, fatty acid metabolism, esophageal gland secretion, and parasitism (Bargmann, 2006; de Almeida Barros et al., 2012; Holden-Dye and Walker, 2011; Perry, 1996; Quentin et al., 2013; Vanholme et al., 2004; Watts and Ristow, 2017).

In *M. incognita*, *odr-1* mRNA was localized in the cell bodies of amphidial neurons and phasmids; and RNAi mediated knockdown of *odr-1, odr-3, tax-2,* and *tax-4* resulted in defective chemotaxis toward volatile and non-volatile compounds (Shivakumara et al., 2019). Major downregulation of all these genes clearly substantiates the fact that the nematode sensory perception was disturbed in the presence of fluensulfone, which was also recorded previously (Kearn et al., 2014; Oka and Saroya, 2019). However, *tax-2* and *tax-4* was upregulated at higher concentrations for longer time of exposure, which can be attributed by some possible feedback mechanism(s) through which the organism try to restore the physiological setbacks (Swain et al., 2010). The OSMotic avoidance abnormal family member 9 (OSM-9) protein coding gene, *osm-9* is expressed in the sensory neurons being involved in taste adaptation, sensory plasticity, and detection of odorants in *C. elegans* (Jansen et al., 2002). In the present study, *osm-9* showed considerable downregulation for all the treatments. The sensory specific G-protein γ subunit, *gpc-1*; G-protein subunits *gpa-11* and *gpa-13*; and the GPCR kinase *grk-2* showed downregulation at 5 hr post exposure for all the three concentrations, but was slightly upregulated for 50 and 100 ppm concentrations at 10 hr. Previous results have shown that *gpa-11* and *gpa-13* regulate life span and navigation in *C. elegans* (Bargmann, 2006; Lans and Jansen, 2007); and *grk-2* loss-of-function strains were egg laying-defective having low levels of serotonin or 5-hydroxytryptamine (5-HT) (Wang et al., 2017). Studies with 5-HT receptor antagonist on *G. pallida* and *C. elegans* indicated possible interaction of fluensulfone with 5-HT signaling to affect stilet thrusting and pharyngeal pumping (Kearn et al., 2014), and this interaction may be interfered by GPCR kinase *grk-2* activity. Perturbation of all these GPCR receptors and subunits possibly hacked the normal physiological processes leading to abnormalities in nematode’s feeding, way-finding, egg laying, and development. However, the differential transcriptional regulation varying with chemical concentration and time of exposure, as observed here, may be intervened by a network of protective and compensatory mechanisms to mitigate the toxicological effects (Bundy et al., 2008; Swain et al., 2010).

Previous studies showed that exposure of *M. incognita* and *M. javanica* to sublethal concentrations of fluensulfone reduced the number of juveniles attracted to root tips resulting in reduced galling (Oka and Saroya, 2019). The esophageal gland specific genes and CWMEs accredit the host finding, migration inside root, development and maintenance of feeding site in *Meloidogyne* spp. (Ding et al., 2000; Shivakumara et al., 2017). As observed here, suppression of all the CWME genes by fluensulfone may collectively result in reducing the
host finding and parasitic potential of the nematode species. However, the msp genes tested here were only downregulated at 5 hr post exposure to 10 ppm of the chemical; but were upregulated in all other time-concentration combinations that might be due to complex crosstalk between the pharyngeal gland genes and CWMEs shaping the nematode’s parasitic potential (Shivakumara et al., 2016, 2017). Fluensulfone treatment also affects the fatty acid metabolism, β-oxidation, and PUFA fractionation processes in nematodes resulting in elevated lipid reserve in the treated worms (Kearn et al., 2017). In C. elegans, fat-6 encodes for stearoyl-coA desaturases that catalyze biosynthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acids and is required for large-sized lipid droplet formation (Brock et al., 2007; Shi et al., 2013). The MUFAs are the most abundant constituent of phospholipids and triglycerides (Enoch et al., 1976), and are important mediators for membrane fluidity and signal transduction (Ntambi, 2004). Downregulation of fat-6, as seen in the present study, may result in decreased utilization of the stored fat reducing the phospholipid and triglyceride formation. Similar suppression of fatty acid elongation gene elo-2 might also aid in reducing the PUFA fractionation into these products (Kniazeva et al., 2003). However, for all the three concentrations fat-6 and elo-2 were upregulated at 10 hr post exposure. This variability may be brought by the complex nexus of gene regulation pattern underlying the fat metabolism process and the response of fat metabolism pathways to environmental toxicants (Brock et al., 2007; Hermsen et al., 2012). Further attenuation of acs-2, mdt-15, nhr-49, ech-5, and ech-6 genes signifies the possible disruption of β-oxidation process (Ashrafi, 2007). As a consequence, the fluensulfone treated worms possibly possess an elevated level of lipid reserve for inability to breakdown and utilize fat simultaneously affecting the ion channel regulation and membrane transfer activity. The impaired energy metabolism (Kearn, 2015) thus plausibly a resultant effect of reduced phospholipid and triglyceride content at cellular level.

The neuropeptides, acetylcholine, FLPs, and NLPs coordinate many crucial aspects of nematode physiology and behaviour (McCoy et al., 2014; McVeigh et al., 2006, 2008; Rand, 2007). In the present study, notably all the neuropeptidergic genes were repressed after fluensulfone treatment. FLP-12, FLP-14, FLP-16, and FLP-18 were identified in root-knot nematodes to coordinate host recognition, parasitism, and development (Kumari et al., 2017; Papolu et al., 2013). Two neuropeptide like protein coding genes, nlp-3 and nlp-12 were found to coordinate locomotion, development, and parasitic ability in M. incognita (Dash et al., 2017). Further, in planta RNAi silencing of M. incognita acetylcholinesterase genes, ace-1 and ace-2, also resulted in reduced galling and parasitism (Cui et al., 2017). These results suggest that fluensulfone might impair the normal activity of these neuropeptides leading to defective developmental, host recognition, locomotion, and parasitism in the nematode. Besides, ace-1 and ace-2 encode for class A and class B acetylcholinesterases, and also regulate nematode locomotion (Combes et al., 2003). Hence, the data suggest that fluensulfone possibly act on acetylcholinesterase, but how it differs from the activity of organophosphates and carbamates is yet to be understood.

In conclusion, our results validate that the nematocidal effects of fluensulfone modulate and impair several checkpoints of nematode biology including chemosensation, esophageal gland secretion, fatty acid metabolism, β-oxidation, polyunsaturated fatty acid (PUFA) fractionation, and neurotransmission at transcriptional level. The observed changes in neuropeptidergic gene expression suggest that the chemical interferes with the regular functioning of neuropeptides, which might be a major cause behind the biological anomalies. Additionally, possibilities of engagement of single gene in different biological functions cannot also be overruled (Chew et al., 2018; Shivakumara et al., 2016; Zhang et al., 2013), which can be true for fluensulfone treatment. However, the study does not include any comparison of effects of fluensulfone with other groups of chemical nematicides, and all the possible molecular targets (studied here) affected by the chemical have been discussed. Presumably, the multidimensional effect of this nematicide is achieved by direct action on the genes and/or pathways governing various physiological functions; or its action on the regulatory receptors and neuropeptides creating a functional imbalance of the downstream gene pool in an anti-narrow direction.

References

Ashrafi, K. 2007. Obesity and the regulation of fat metabolism, In WormBook, (Ed.), The C. elegans Research Community, doi: 10.1895/wormbook.1.130.1.
Bargmann, C. I. 2006. Chemosensation in C. elegans, In WormBook, (Ed.), The C. elegans Research Community, doi: 10.1895/wormbook.1.123.1.
Berg, R. H., Fester, T. and Taylor, C. G. 2009. “Development of the root-knot nematode feeding cell”,
Effect of fluensulfone on different M. incognita genes: Hada et al.

In Berg, R. H. and Taylor, C. G. (Eds), Cell Biology of Plant Nematode Parasitism, Springer, Berlin and Heidelberg, pp. 115–52.

Blanchard, A., Guégnard, F., Charvet, C. L., Crisford, A., Courtot, E., Sauvé, C., Harmache, A., Duguet, T., O’Connor, V., Castagnone-Sereno, P. and Reaves, B. 2018. Deciphering the molecular determinants of cholinergic anthelmintic sensitivity in nematodes: when novel functional validation approaches highlight major differences between the model and parasitic species. PLoS Pathogens 14:e1006996.

Bridge, J. 1996. Nematode management in sustainable and subsistence agriculture. Annual Review of Phytopathology 34:201–25.

Brock, T. J., Browse, J. and Watts, J. L. 2007. Fatty acid desaturation and the regulation of adiposity in Caenorhabditis elegans. Genetics 176:865–75.

Bundy, J. G., Sidhu, J. K., Rana, F., Spurgeon, D. J., Svendsen, C., Wren, J. F., Stürzenbaum, S. R., Morgan, A. J. and Kille, P. 2008. Systems toxicology’ approach identifies coordinated metabolic responses to copper in a terrestrial non-model invertebrate, the earthworm Lumbricus rubellus. BMC Biology 6:1–25.

Chaudhary, S., Dutta, T. K., Tyagi, N., Shivakumara, T. N., Papolu, P. K., Chobhe, K. A. and Rao, U. 2019. Host-induced silencing of Mi-msp-1 confers resistance to root-knot nematode Meloidogyne incognita in eggplant. Transgenic Research 28:327–40.

Chew, Y. L., Grundy, L. J., Brown, A. E. X., Beets, I. and Schafer, W. R. 2018. Neuropeptides encoded by nip-49 modulate locomotion, arousal and egg-laying behaviours in Caenorhabditis elegans via the receptor SEB-3. Philosophical Transactions of the Royal Society B 373:20170368.

Combes, D., Fedon, Y., Toutant, J. P. and Arpagaus, M. 2003. Multiple ace genes encoding acetylcholinesterases of Caenorhabditis elegans have distinct tissue expression. European Journal of Neuroscience 18:497–512.

Cui, R., Zhang, L., Chen, Y., Huang, W., Fan, C., Wu, Q., Peng, D., da Silva, W. and Sun, X. 2017. Expression and evolutionary analyses of three acetylcholinesterase genes (Mi-ace-1, Mi-ace-2, Mi-ace-3) in the root-knot nematode Meloidogyne incognita. Experimental Parasitology 176:75–81.

Dashi, M., Dutta, T. K., Phani, V., Papolu, P. K., Shivakumara, T. N. and Rao, U. 2017. RNAi-mediated disruption of neuropeptide genes, nip-3 and nip-12, cause multiple behavioral defects in Meloidogyne incognita. Biochemical and Biophysical Research Communications 490:933–40.

de Almeida Barros, A. G., Liu, J., Lemieux, G. A., Mullaney, B. C. and Ashrafi, K. 2012. Analyses of C. elegans fat metabolic pathways. Methods in Cell Biology 107:383–407.

Decraemer, W. and Hunt, D. J. 2006. “Structure and classification”, In Perry, R. N. and Moens, M. (Eds), Plant Nematology, CABI Publishing, Wallingford, pp. 3–32.

Ding, X., Shields, J. P., Allen, R. I. and Hussey, R. S. 2000. Molecular cloning and characterization of a venom allergen AG5-like cDNA from Meloidogyne incognita. International Journal for Parasitology 30:77–81.

Dutta, T. K., Khan, M. R. and Phani, V. 2019. Plant-parasitic nematode management via biofumigation using brassica and non-brassica plants: current status and future prospects. Current Plant Biology 17:17–32.

Elling, A. A. 2013. Major emerging problems with minor Meloidogyne species. Phytopathology 103:1092–102.

Enoch, H. G., Catalá, A. and Strittmatter, P. 1976. Mechanism of rat liver microsomal stearyl-CoA desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. Journal of Biological Chemistry 251:5095–103.

Faske, T. R. and Hurd, K. 2015. Sensitivity of Meloidogyne incognita and Rotylenchulus reniformis to fluopyram. Journal of Nematology 47:316–21.

Fleming, T. R., Maule, A. G. and Fleming, C. C. 2017. Chemosensory responses of plant parasitic nematodes to selected phytochemicals reveal long-term habituation traits. Journal of Nematology 49:462–71.

Hermsen, S. A., Pronk, T. E., van den Brandhof, E. J., van der Ven, L. T. and Piersma, A. H. 2012. Concentration-response analysis of differential gene expression in the zebrafish embryotoxicity test following flusilazole exposure. Toxicological Sciences 127:303–12.

Holden-Dye, L. and Walker, R. J. 2011. Neurobiology of plant parasitic nematodes. Invertebrate Neuroscience 11:9–19.

Huang, G. Z., Gao, B., Maier, T., Allen, R., Davis, E. L., Baum, T. J. and Hussey, R. S. 2003. A profile of putative parasitism genes expressed in the esophageal gland cells of the root-knot nematode Meloidogyne incognita. Molecular Plant Microbe Interaction 16:376–81.

Jansen, G., Weinkove, D. and Plasterk, R. H. 2002. The G-protein γ subunit gpc-1 of the nematode C. elegans is involved in taste adaptation. The EMBO Journal 21:986–94.

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. and Perry, R. N. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology 14:946–61.

Kearn, J., Ludlow, E., Dillon, J., O’Connor, V. and Holden-Dye, L. 2014. Fluensulfone is a nematicide with a mode of action distinct from anticholinesterases and macrocyclic lactones. Pesticide Biochemistry and Physiology 109:44–57.

Kearn, J. 2015. Mode of action studies on the nematicide fluensulfone. Doctoral dissertation, University of Southampton.

Kearn, J., Lilley, C., Urwin, P., O’Connor, V. and Holden-Dye, L. 2017. Progressive metabolic impairment
underlies the novel nematicidal action of fluensulfone on the potato cyst nematode Globodera pallida. Pesticide Biochemistry and Physiology 142:83–90.

Kim, K. H., Kabir, E. and Jahan, S. A. 2017. Exposure to pesticides and the associated human health effects. Science of Total Environment 575:525–35.

Kniazeva, M., Sieber, M., McCauley, S., Zhang, K., Watts, J. L. and Han, M. 2003. Suppression of the ELO-2 FA elongation activity results in alterations of the fatty acid composition and multiple physiological defects, including abnormal ultradian rhythms, in Caenorhabditis elegans. Genetics 163:159–69.

Kumari, C., Dutta, T. K., Chaudhary, S., Banakar, P., Papolu, P. K. and Rao, U. 2017. Molecular characterization of FMRF amide-like peptides in Meloidogyne graminicola and analysis of their knockdown effect on nematode infectivity. Gene 619:50–60.

Lahm, G. P., Desaeger, J., Smith, B. K., Pahutski, T. F., Rivera, M. A., Meloro, T., Kucharczyk, R., Lett, R. M., Daly, A., Smith, B. T., Cordova, D., Thoden, T. and Wiles, J. A. 2017. The discovery of fluazaindolizine: a new product for the control of plant parasitic nematodes. Bioorganic & Medicinal Chemistry Letters 27:1572–5.

Lans, H. and Jansen, G. 2007. Multiple sensory G proteins in the olfactory, gustatory and nociceptive neurons modulate longevity in Caenorhabditis elegans. Developmental Biology 303:474–82.

Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2ΔΔCt method. Methods 25:402–8.

Mantelin, S., Thorpe, P. and Jones, J. T. 2017. Translational biology of nematode effectors or, to put it another way, functional analysis of effectors – what’s the point? Nematology 19:251–61.

McCoy, C. J., Atkinson, L. E., Zamanian, M., McVeigh, P., Day, T. A., Kimber, M. J., Marks, N. J., Maule, A. G. and Mousley, A. 2014. New insights into the FLPeric complements of parasitic nematodes: informing deorphanisation approaches. EuPA Open Proteomics 3:262–72.

McVeigh, P., Geary, T. G., Marks, N. J. and Maule, A. G. 2006. The FLP-side of nematodes. Trends in Parasitology 22:385–96.

McVeigh, P., Alexander-Bowman, S., Veal, E., Mousley, A., Marks, N. J. and Maule, A. G. 2008. Neuropeptide-like protein diversity in phylum Nematoda. International Journal for Parasitology 38:1493–503.

Ntambi, J. 2004. Regulation of stearyl-CoA desaturases and role in metabolism. Progress in Lipid Research 43:91–104.

Oka, Y., Beson, M. and Barazani, A. 2008. MCW-2: a ‘true’ nematicide belonging to the fluoroalkenyl group. Proceedings of the 5th International Congress of Nematology; Brisbane, pp. 313–4.

Oka, Y., Shuker, S. and Tkachi, N. 2009. Nematicidal efficacy of MCW-2, a new nematicide of the fluoroalkenyl group, against the root-knot nematode Meloidogyne javanica. Pest Management Science 65:1082–9.

Oka, Y., Shuker, S. and Tkachi, N. 2012. Systemic nematicidal activity of fluensulfone against the root-knot nematode Meloidogyne incognita on pepper. Pest Management Science 68:268–75.

Oka, Y., Shuker, S. and Tkachi, N. 2013. Influence of some soil environments on the nematicidal activity of fluensulfone against Meloidogyne javanica. Pest Management Science 69:1225–34.

Oka, Y. 2014. Nematicidal activity of fluensulfone against some migratory nematodes. Pest Management Science 70:1850–8.

Oka, Y. and Saroya, Y. 2019. Effect of fluensulfone and fluopyram on the mobility and infection of second-stage juveniles of Meloidogyne incognita and M. javanica. Pest Management Science 75:2095–106.

Papolu, P. K., Gantasala, N. P., Kamaraju, D., Banakar, P., Sreevathsa, R. and Rao, U. 2013. Utility of host delivered RNAi of two FMRF amide like peptides, fpl-14 and fpl-18, for the management of root knot nematode, Meloidogyne incognita. PLoS One 8:e80603.

Perry, R. N. 1996. Chemoreception in plant parasitic nematodes. Annual Review of Phytopathology 34:181–99.

Phani, V., Somvanshi, V. S., Shukla, R. N., Davies, K. G. and Rao, U. 2018. A transcriptomic snapshot of early molecular communication between Pasteuria penetrans and Meloidogyne incognita. BMC Genomics 19:850.

Ploeg, A., Stoddard, S. and Becker, J. O. 2019. Control of Meloidogyne incognita in sweetpotato with fluensulfone. Journal of Nematology 51:e2019–18.

Quentin, M., Abad, P. and Favery, B. 2013. Plant parasitic nematode effectors target host defense and nuclear functions to establish feeding cells. Frontiers in Plant Science 4:53.

Rand, J. B. 2007. “Acetylcholine”, In WormBook, (Ed), The C. elegans Research Community, doi: 10.1895/ wormbook1.131.1.

Shi, X., Li, J., Zou, X., Greggain, J., Rødkær, S. V., Færgeman, N. J., Liang, B. and Watts, J. L. 2013. Regulation of lipid droplet size and phospholipid composition by stearoyl-CoA desaturase. Journal of Lipid Research 54:2504–14.

Shirley, A. M., Noe, J. P., Nyczepir, A. P., Brannen, P. M., Shirley, B. J. and Jagdale, G. B. 2019. Effect of spirotetramat and fluensulfone on population densities of Mesocricitonema xenoplax and Meloidogyne incognita on peach. Journal of Nematology 51:e2019–12.

Shivakumara, T. N., Chaudhary, S., Kamaraju, D., Dutta, T. K., Papolu, P. K., Banakar, P., Sreevathsa, R., Singh, B., Manjaiah, K. M. and Rao, U. 2017. Host-induced silencing of two pharyngeal gland genes confers transcriptional alteration of cell wall-modifying enzymes of Meloidogyne incognita vis-à-vis perturbed nematode infectivity in eggplant. Frontiers in Plant Science 8:473.
Effect of fluensulfone on different *M. incognita* genes: Hada et al.

Shivakumara, T. N., Papolu, P. K., Dutta, T. K., Kamaraju, D., Chaudhary, S. and Rao, U. 2016. RNAi-induced silencing of an effector confers transcriptional oscillation in another group of effectors in the root-knot nematode, *Meloidogyne incognita*. Nematology 18:657–70.

Shivakumara, T. N., Dutta, T. K., Chaudhary, S., von Reuss, S. H., Williamson, V. M. and Rao, U. 2019. Homologs of *Caenorhabditis elegans* chemosensory genes have roles in behavior and chemotaxis in the root-knot nematode *Meloidogyne incognita*. Molecular Plant Microbe Interaction 32:876–87.

Slomczynska, U., South, M. S., Bunkers, G., Edgecomb, D., Wyse-Pester, D., Selness, S., Ding, Y., Christiansen, J., Ediger, K., Miller, W., Charumilind, P., Hartmann, G., Williams, J., Dimmic, M., Shortt, B., Haakenson, W., Wideman, A., Crawford, M., Hresko, M. and McCarter, J. 2014. Tioxazafen: a new broad-spectrum seed treatment nematicide. 13th IUPAC Int Congr Pesticide Chemistry, AGRO-39, San Francisco, CA, August 10–14.

Swain, S., Wren, J. F., Stürzenbaum, S. R., Kille, P., Morgan, A. J., Jager, T., Jonker, M. J., Hankard, P. K., Svendsen, C., Owen, J. and Hedley, B. A. 2010. Linking toxicant physiological mode of action with induced gene expression changes in *Caenorhabditis elegans*. BMC System Biology 4:32.

US-EPA 2008. Fenamiphos: amendment to use deletion and product cancellation order. Federal Register 73:75097–9.

US-EPA 2009. Ethoprop: notice of receipt of request to voluntarily amend a pesticide registration to terminate use. Federal Register 74:25237–9.

Vanholme, B., De Meutter, J., Tytgat, T., Van Montagu, M., Coomans, A. and Gheysen, G. 2004. Secretions of plant-parasitic nematodes: a molecular update. Gene 332:13–27.

Waldo, B. D., Grabau, Z. J., Mengistu, T. M. and Crow, W. T. 2019. Nematicide effects on non-target nematodes in bermudagrass. Journal of Nematology 51:e2019–09.

Wang, J., Luo, J., Aryal, D. K., Wetsel, W. C., Nass, R. and Benovic, J. L. 2017. G protein-coupled receptor kinase-2 (GRK-2) regulates serotonin metabolism through the monoamine oxidase AMX-2 in *Caenorhabditis elegans*. Journal of Biological Chemistry 292:5943–56.

Watts, J. L. and Ristow, M. 2017. Lipid and carbohydrate metabolism in *Caenorhabditis elegans*. Genetics 207:413–46.

Whitehead, A. G. and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology 55:25–38.

Wram, C. L. and Zasada, I. A. 2019. Short-term effects of sublethal doses of nematicides on *Meloidogyne incognita*. Phytopathology 109:1605–13.

Zhang, Y., Zou, X., Ding, Y., Wang, H., Wu, X. and Liang, B. 2013. Comparative genomics and functional study of lipid metabolic genes in *Caenorhabditis elegans*. BMC Genomics 14:164.
Table S1. Homology parameters of unannotated genes used in this study.

| Sl | Primers name  | Gene name (accession number)     | Primers Sequences (5′→3′)                          | Amplicon length (bp) | Identity (%) | Score   | E-value         | Purpose |
|----|---------------|----------------------------------|----------------------------------------------------|---------------------|--------------|---------|----------------|---------|
| 1  | RT miOSM-9 F  | M. incognita osm-9 homologue    | CATGCCTGAAGATTTGGAAG GGTCCAGGATTAGCACCATAC         | 150                 | 100          | 124     | 3.6E−62         | qRT PCR |
|    | RT miOSM-9 R  |                                   |                                                    |                     |              |         |                 |         |
| 2  | RT miGPC-1 F  | M. incognita gpc-1 homologue    | CAACTTCGACAAGAGGCAA CCTATTGTTGGTAATTAAG            | 117                 | 100          | 199     | 1.4E−18         | qRT PCR |
|    | RT miGPC-1 R  |                                   |                                                    |                     |              |         |                 |         |
| 3  | RT mi GPA-11 F|M. incognita gpa-11 homologue   | CGACTACGGGTATTTGTGAC GAAAGTTCGCTACGAACAG           | 143                 | 100          | 58      | 8.2E−23         | qRT PCR |
|    | RT miGPA-11 R |                                   |                                                    |                     |              |         |                 |         |
| 4  | RT miGPA-13 F | M. incognita gpa-13 homologue   | GCTGAAAATTTCAGAGGATGG GCTGATTCACGAGAAGC            | 112                 | 100          | 194     | 1.1E−17         | qRT PCR |
|    | RT miGPA-13 R |                                   |                                                    |                     |              |         |                 |         |
| 5  | RT miGRK-2 F  | M. incognita grk-2 homologue    | CTCAATTCTACGGCAGGAG GGTGCCATAGAGCAG               | 124                 | 100          | 202     | 4.4E−19         | qRT PCR |
|    | RT miGRK-2 R  |                                   |                                                    |                     |              |         |                 |         |
| 6  | RT miFAT-6 F  | M. incognita fat-6 homologue    | GAATTTCTGCAATCGTCCAC CATCACTGTCGTCAC              | 162                 | 100          | 306     | 1.9E−33         | qRT PCR |
|    | RT miFAT-6 R  |                                   |                                                    |                     |              |         |                 |         |
| 7  | RT miACS-2 F  | M. incognita acs-2 homologue    | CCCAACCAGAGGAAAGGATGA CGCTCTTATACCAAGGTCT          | 200                 | 100          | 356     | 4.2E−40         | qRT PCR |
|    | RT miACS-2 R  |                                   |                                                    |                     |              |         |                 |         |
| 8  | RT miMDT-15 F | M. incognita mdt-15 homologue   | CACCTATGACCACAGGTTC CTAACCTCTCTCCACATTCT            | 100                 | 100          | 105     | 2.5E−5          | qRT PCR |
|    | RT miMDT-15 R |                                   |                                                    |                     |              |         |                 |         |
| 9  | RT miNHR-49 F | M. incognita nhr-49 homologue   | GCCAATGGCAGCATTAGAG CCTCGTCTACGGTTTAG              | 179                 | 100          | 294     | 9.4E−32         | qRT PCR |
|    | RT miNHR-49 R |                                   |                                                    |                     |              |         |                 |         |
| 10 | RT miELO-2 F  | M. incognita elo-2 homologue    | CAAACGGATTTGGGTGCTGG GCCGTGATAGGGTAGGAATA          | 148                 | 100          | 64      | 2.2E−26         | qRTPCR |
|    | RT miELO-2 R  |                                   |                                                    |                     |              |         |                 |         |
| 11 | RT miECH-5 F  | M. incognita ech-5 homologue    | CACTAGGGAGGTACGTACA CTTGGAGCTTTTGAGT               | 130                 | 100          | 209     | 4.7E−20         | qRT PCR |
|    | RT miECH-5 R  |                                   |                                                    |                     |              |         |                 |         |
| 12 | RT miECH-6 F  | M. incognita ech-6 homologue    | CGAGGCTCTTGGGAGGGATA GCTTATGAGGGAGGAGG            | 193                 | 100          | 342     | 1.3E−36         | qRT PCR |
|    | RT miECH-6 R  |                                   |                                                    |                     |              |         |                 |         |
Table S2. Assessment of mortality of *M. incognita* J2s at variable concentrations of fluensulfone and time of exposure.

| Fluensulfone concentration (ppm) | Nematode mortality (%) | Remarks |
|----------------------------------|------------------------|---------|
|                                  | 1hr  | 2.5hr | 5hr  | 7.5hr | 10hr | 24hr |              |
| 100                              | 0.86 | 3.47  | 8.62 | 18.51 | 27.78| 100  | Reduced motility and agility observed 0.5hr onward the drug treatment |
| 75                               | 0    | 1.74  | 4.35 | 8.62  | 18.18| 100  | Reduced motility and agility observed 1hr onward the drug treatment |
| 50                               | 0    | 1.74  | 8.08 | 13.79 | 13.79| 100  | Reduced motility and agility observed 1hr onward the drug treatment |
| 25                               | 0    | 0.86  | 2.59 | 8.62  | 11.01| 90.74| Reduced motility and agility observed 4hr onward the drug treatment |
| 10                               | 0    | 0     | 0    | 2.59  | 6.03 | 75.92| Reduced motility and agility observed 4hr onward the drug treatment |
| 5                                | 0    | 0     | 0    | 1.74  | 1.74 | 43.48| Reduced motility and agility observed 7hr onward the drug treatment |
| 1                                | 0    | 0     | 0    | 0     | 1.74 | 29.57| Reduced motility and agility in few worms observed 7–8hr onward the drug treatment |
| Vehicle control (0.05% DMSO)     | 0    | 0     | 0    | 0     | 0    | 0    | No change in motility and agility |