



NemaMetrix

EFFECT OF FENNEL ESSENTIAL OIL ON PHARYNGEAL PUMPING IN *C. elegans*

USING THE SCREENCHIP SYSTEM

PURPOSE

Anthelmintics are a category of drugs used to rid humans and animals of parasitic nematodes (worms). Current anthelmintics have a number of shortcomings, including the increasing development of resistance. New anthelmintics may be discovered by screening libraries of natural products and small molecules, or re-purposing existing FDA-approved drugs. Most existing anthelmintics act on ion channels or neurotransmitter receptors, so determining whether a candidate compound impairs electrical signaling is useful to know early in the screening process. Essential oil of fennel (*Foeniculum vulgare*) has been used traditionally in South America as a worming agent against human infections with the trematode parasite, *Schistosoma mansoni* (1). Using the ScreeningChip platform, we investigated the effect of fennel essential oil (FEO) on *C. elegans*.

METHODS

C. Elegans synchronization and cultivation

Synchronized wild type N2 *C. elegans* were obtained via a bleach synchronization procedure. The resulting L1s were cultivated on plates containing standard nematode growth medium (NGM) seeded with *E. coli* OP50 and allowed to grow at 20°C until they reached the first day of adulthood (2, 3).

Solutions

Stock solutions were prepared as 2 μ L FEO added to 90 μ L DM50. 5 μ L of this stock was added to 1 ml of M9 buffer containing 10 mM 5HT to stimulate pumping (4). The final concentrations in the FEO solution were 106 mg/mL FEO, 0.5% DMSO and 10mM 5HT. The 10mM 5HT solution was made in a 15 mL conical vial by adding 10 ML of M9 to 43 mg 5HT and used within 4 h.

Recording electropharyngeograms (EPGs)

A glass pipette with M9 buffer was used to rinse day 1 adults from a plate. They were transferred to a 1.5 mL Eppendorf tube (Epi tube) and centrifuged for 2 min at 6,000 RPM. The supernatant was removed and replaced with fresh M9 and the worms were spun down again. The supernatant was removed again and replaced with M9 containing 10 mM 5HT. Worms were incubated for 20 min to activate pharyngeal pumping. After incubation they were spun down for another 2 min at 6,000 RPM and the supernatant was removed and replaced with M9 containing 10 mM 5HT with either 0.5% DMSO (control group) or 0.5% DMSO and 106 mg/L FEO (experimental group). Pharyngeal pumping frequency was measured in EPGs from 10 worms in each group using the NemaMetrix ScreenChip system, using methods described in the ScreenChip User Guide (5). Each EPG recording was 2 to 4 min in duration and the experiment was replicated in triplicate on different days. EPG recordings were started ~5 min after the onset of FEO exposure and continued for an additional 45 min.



NemaMetrix, Inc.
44 W 7th Ave
Eugene, OR 97401
1-844-663-8749
nemamatrix.com

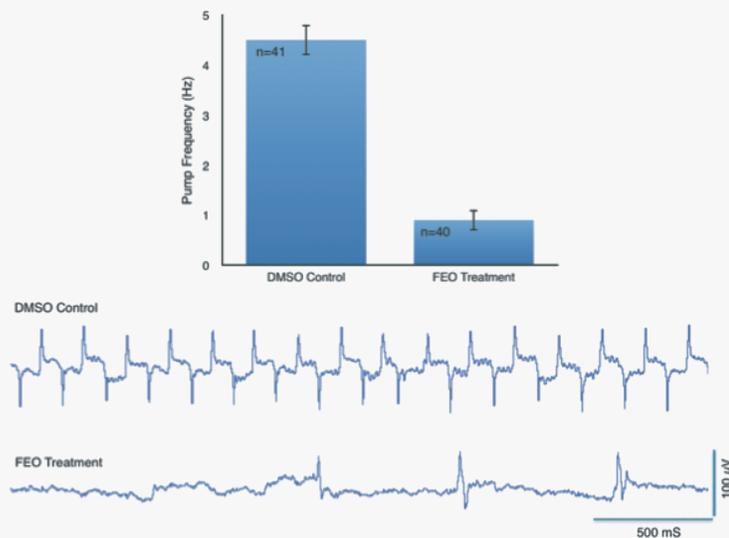
RESULTS

The table below presents pumping frequency data for the two groups.

Pump Frequency (hz)

	DMSO Control	Fennel Essential Oil Treatment
Mean +/- SEM	4.5 +/- 0.30	0.9 +/- 0.22
n (worms)	41	40

Pump frequency was significantly reduced in the FEO treatment group compared to solvent controls (*; $t(81) = 10.135$, $p < 0.0001$, 2-tailed T-test). These data are also shown below as histograms (mean + SEM), with a representative EPG recording from each group. The time and voltage scale bars apply to both recordings. In addition to reduced pump frequency, FEO caused aberrant EPG waveforms.



CONCLUSION

Fennel essential oil has been shown to have anthelmintic properties against the human parasite, *S. mansoni* (1). In the present study, we determined that FEO at

106 mg/mL (similar to the effective concentration in *S. mansoni*) inhibited pharyngeal pumping in *C. elegans*, consistent with anthelmintic activity. FEO contains at least 10 chemical constituents, which may be more effective in combinations than when applied singly (1).

These experiments support the utility of the Screen-Chip system for testing natural products for anthelmintic activity in *C. elegans*. Microfluidic EPG recordings from the human hookworm, *Ancylostoma ceylanicum*, have likewise confirmed vermicide activity in extracts of bitter melon, *Momordica charantia*, which is used traditionally as a worming decoction in Haiti (6). EPG recordings provide results more rapidly than traditional endpoints such as worm paralysis or egg production and allow researchers to rapidly pursue mode-of-action experiments using *C. elegans*.

REFERENCES

- (1) Wakabayashi, KAL et al. 2015. Anthelmintic effects of the Essential Oil of Fennel (*Foeniculum vulgare* Mill., Apiaceae) against *Schistosoma mansoni*. *Chemistry & Biodiversity* 12:1105-1114
- (2) Stiernagle T. 2006. Maintenance of *C. elegans*. *Wormbook*, http://222.wormbook.org/chapters/www_strainmaintain/strainmaintain.html
- (3) Porta-de-la-Riva, M et al. 2012. Basic *Caenorhabditis elegans* methods: synchronization and observation *J Vis Exp* 64:4019
- (4) Lockery SR et al. 2012. A microfluidic device for whole-animal drug screening using electrophysiological measures in the nematode *C. elegans* *Lab Chip* 12:2211-2220
- (5) NemaMetrix Screenchip100 User Guide. www.nemamatrix.com
- (6) Wolpert BJ et al. 2008. Plant vermicides of Haitian Vodou show in vitro activity against larval hookworm. *J Parasitol* 94:1155-60; ML Keaney, A MacIntyre, K Goggins, KJ Robinson, WM Roberts, JC Weeks & JG Hawdon, in preparation

