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Caenorhabditis elegans as an *in vitro* model for the evaluation of anthelmintic activities of essential oil blends.

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Introduction

Parasitism is a major concern in livestock, involving production losses, pathologies or even the death of animals. In addition, the development of resistance of worms to classic molecules encourages veterinarians and breeders to modify their treatment protocols but also the search for innovative alternatives.

Plant extracts and more particularly essential oils (EOs) are recognized for their *in vitro* and *in vivo* anthelmintic activities. This property, in addition to antioxidant, anti-inflammatory and immunomodulatory activities, make them good candidates for the management of parasitism in farms. Specific *in vitro assays* must however be carried out in order to select the most active essential oil blend, against the targeted helminths.

Most intestinal parasites requires a developmental stage in the host, which requires the sacrifice of animals, even for the performance of *in vitro* assays. In order to limit this unethical practice and reduce developmental costs, we propose here a model based on the *Ceanorhabditis elegans* nematode in order to evaluate the effectiveness of mixtures of essential oils (EOBs).

Context and objectives of the study

- *Caenorhabditis elegans* is a round worm belonging to clade V, like several parasites of interest such as *Haemonchus contortus, Oesophagostomum dentatum* or *Teladorsagia circumcincta,* affecting production animals.
- *C. elegans* feeds on bacteria and its complete life cycle can be achieved without a host (Fig 1).
- Its small size (1mm), its transparency and the existence of many mutants makes it an ideal laboratory model.
- Historically used to understand cell and embryonic development, its use is now developing in many fields (pharmaceuticals, cosmetics, comprehension of infectious diseases…).

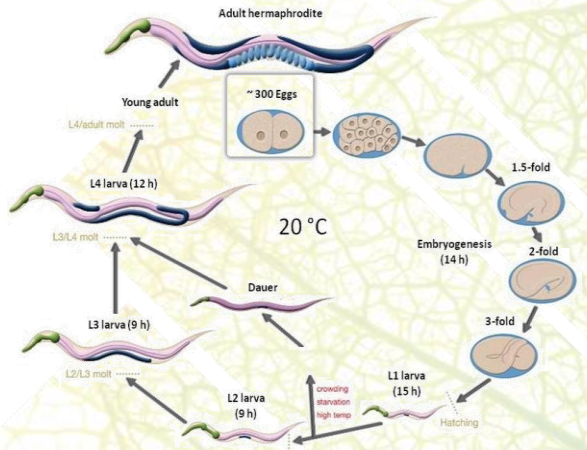

Figure 1 : Development of *C. elegans* at 20°C

STEP 1

In vitro evaluation of essential oils and proposed blends on *C. elegans,* using toxicity assay (L1 and L4) and egg hatching inhibition assay.

Methodology is developed in liquid media and adapted to essential oils.

STEP 2

In vitro evaluation of selected essential oil blends on *Haemonchus contortus* using hatching and motility inhibition assays.

STEP 3

In vivo evaluation of the most promising essential oil blend after formulation, on lambs infected with *H. contortus.*

Figure 2 : Progress of the study allowing the creation and selection of formulated essential oils blends with *in vivo* anthelmintic activities.

- Complete development cycle of *C. elegans* at the laboratory in solid or liquid media allows the setting up of assays adapted to lipophilic essential oils (EOs) (Fig 2).
- Due to it small size *C. elegans* fits into microplate wells, allowing the evaluation of a large number of candidates.
- According to the literature, extract with nematocidal activities on *C. elegans* are fifteen times more likely to be efficient on parasites of interest (Burns, A. R., et al. 2015).

  → First step of the process should allow a screening of EOs and a selection of active EOBs. Five essential oils (*Cinnamomum cassia* (CEO), *Origanum vulgare* (OEO), *Satureja hortensis* (SaEO), *Thymus vulgaris* (TEO) and *Syzygium aromaticum* (SyEO)) and four blends (EOB1 – 4) are evaluated.

  → *In vitro* evaluation followed by on farm trials using *H. contortus* should confirm effectiveness and inform on the bioavaibility of formulated EOBs.

Results

STEP 1 : EOs and EOBs are evaluated for their toxicity toward *C. elegans.*

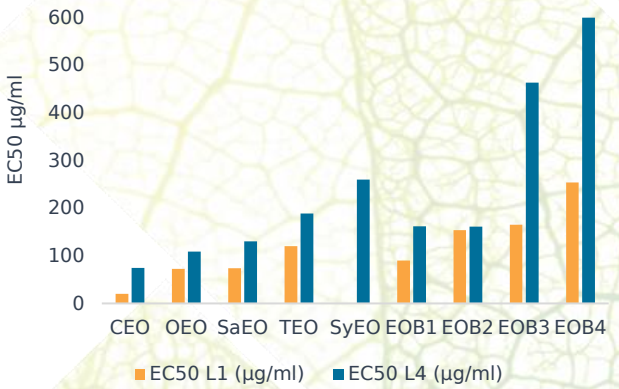

Figure 3 : Evaluation of the toxicity of EOs and Blends on *C. elegans,* after a 24h treatment on L1 or L4 worms, in liquid media. EC_{50} are presented in µg/ml.

All tested EOs and blends develop anthelmintic activity against *C. elegans.* Cinnamon is the most toxic EO, with EC_{50} of 20.2 µg/ml and 74.7 µg/ml on L1 and L4 worms respectively. EOB1 and EOB2 present similar anthelmintic activities and are selected for the egg hatching assay.

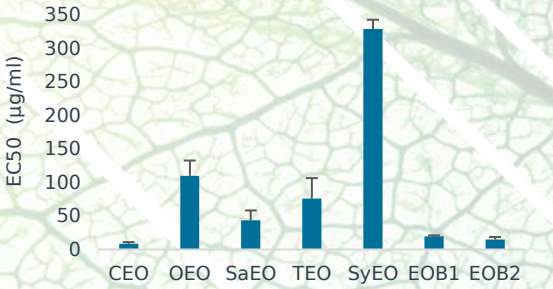

Figure 4 : Evaluation of egg hatching inhibition activity of EOs, EOB1 and EOB2 on *C. elegans,* after a 24h treatment in liquid media. EC_{50} are presented in µg/ml.

Again, CEO is the most active, inhibiting egg hatching by 50% at the concentration of 8.5 µg/ml. This assay didn't allow us to significantly distinguish EOB1 from EOB2 (EC_{50}= 19.6 and 15.0 µg/ml respectively). Both blends are tested against *H. contortus, in vitro*.

STEP 2 : EOBs are evaluated against *H. contortus.*

Table 1 : EOB1 and EOB2 are tested *in vitro* on the parasitic nematode *H. contortus* to evaluate their capacity to reduce egg hatching and larval motility. EC_{50} are given in µg/ml.

	EOB1	EOB2
EC_{50} Egg hatching inhibition	92	199
EC_{50} Larval motility inhibition	715	N.D.

N.D. = not determined.

EOB1 is the most efficient blend against the parasitic nematode *H. contortus*, reducing egg hatching and larval motility. Effect of EOB2 on larval motility could not be evaluated using this assay due to a non linear response.

Conclusion and perspectives

Assays carried out on *C. elegans* allowed us to identify EOB1, active against *H. contortus*, a parasitic nematode affecting ruminants.

Results obtained using the assays are consistent on both nematodes, which supports the use of *C. elegans* for the pre-screening of natural extracts. The use of this model has many advantages, summarized in table 2.

The predictivity of the *C. elegans* model needs now to be validated on other parasites, and thanks to *in vivo* trials. Its development may ultimately reduce the cost of research and offer an alternative to animal testing in the future.

Table 2 : Main advantages and disadvantages of using *C. elegans* as a model for the research of new anthelmintics

Advantages	Inconvenients
Small size / adaptability to HCS	Absence of characteristics specific to parasitism
Transparency	Target can be modified or absent
Short generation time	Post-traductional modification of proteins can differ
Cycle completed without host	
Safe for operator	
Facilitated mutagenesis	

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