In vivo anthelmintic activity of terpenes and essential oils in small ruminants

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ABSTRACT

Terpenes and essential oils (EsOi) have a wide range of biological activities that could be explored in veterinary sciences. In this sense, their use has been proposed as a strategy to overcome the growing populations of gastrointestinal nematodes (GIN) resistant to the traditional anthelmintic (AH) drugs. In the present review, we analysed eleven scientific manuscripts aimed to evaluate the AH activity of terpene-rich plants (TRP) and EsOi in small ruminants by using the in vivo approach. The sheep species was used in the 81% of works. Brazil is the country leading this research line, followed by the United States of America and Benin. All the analysed manuscripts employed the faecal egg count reduction test, while five manuscripts employed the controlled test—or trial—methodology. The activity of TRP and EsOi on the faecal excretion of GIN eggs was variable, showing values between zero to 97%. Two manuscripts reported a reduction in the size of male GIN, as well as the fecundity of female GIN after administration of EsOi. It would be worth to perform more works aimed to understand the relationships between plants, their secondary metabolites and the ruminants consuming them. Through the understanding of such interactions, we will be allowed to use these natural products as elements that could contribute to improve the nutrition and health of sheep and goats under different productive systems.

Keywords: Ethnoveterinary; gastrointestinal nematodes; goat; parasite egg count; parasitic diseases; plant secondary metabolites; sheep (Sources: CAB, MeSH)

RESUMEN

Los terpenos y aceites esenciales (AcEs) poseen un amplio espectro de actividades biológicas que pueden ser exploradas en las ciencias veterinarias. En este sentido, su uso ha sido propuesto como una estrategia para enfrentar las crecientes poblaciones de nematodos gastrointestinales (NGI) resistentes a los antihelmínticos (AH) tradicionales. En la presente revisión analizamos 11 manuscritos científicos que, mediante la utilización del enfoque in vivo, evaluaron el potencial AH de plantas ricas en terpenos (PRT) o AcEs en pequeños rumiantes. La especie ovina fue utilizada en el 81% de los trabajos. Brasil es el país que lidera esta línea de investigación seguido de los Estados Unidos de América y Benin. Todos los manuscritos analizados utilizaron la prueba de reducción...
del conteo fecal de huevos de NGI, mientras que cinco manuscritos emplearon la metodología del test —o prueba— controlado(a). La actividad de las PRT y los AcEs sobre la excreción de huevos de NGI en las heces fue variable, reportándose valores que oscilaron desde un efecto nulo hasta un 97%. Dos trabajos reportaron una reducción en el tamaño de los NGI machos adultos y en la fecundidad de las hembras tras la administración de AcEs. Resulta necesario generar más trabajos que se dirijan a entender las interacciones entre las plantas, sus metabolitos secundarios y los rumiantes que las consumen. Comprender dichas interacciones nos permitirá utilizar estos productos naturales como elementos que ayuden a mejorar la nutrición y la sanidad de ovinos y caprinos en diferentes sistemas productivos.

**Palabras clave:** Conteo de huevos de parásitos; enfermedades parasitarias; etnoveterinaria; nematodos gastrointestinales; cabras; metabolitos secundarios de plantas; ovejas (Fuentes: CAB, MeSH).

**INTRODUCTION**

**Integrated control of gastrointestinal nematodes in small ruminants**

Any sheep/goat livestock system based on pasture utilization has the unavoidable consequence of face gastrointestinal nematodes (GIN). Nowadays, the infection caused by GIN continues to be considered as the main health drawback for small ruminant flocks, which negatively impacts the viability and profitability of these livestock systems (1,2). The latter situation is worsened with the triggering of GIN strains resistant to traditional anthelmintics (AH), even toward the recently introduced active principles (5-8). According with some estimates, losses associated with GIN infection are —in average— 151,206, and 86 million euros in dairy sheep, meat sheep, and dairy goat industries, respectively (9). Likewise, the European Union reported losses averaging 1.8 billion euros associated with AH resistance (10). In the search for non-pharmacological and sustainable strategies, the concept of ‘integrated control of gastrointestinal nematodes’ has been embedded. This compendium of strategies is inherently complementary (allowing the possibility for combinations at the farm level) and is aimed to favor the control over GIN eradication. Within this range of possibilities, it is worth to mention the rational use of AH (11,12), nutritional supplementation (13,14), genetic selection (15,16), immunization (17,18), copper oxide wire particles (19,20), rotational grazing (21), bio control (22,23), and the elucidation of AH properties in many plant species (24,25,26).

**Plant secondary metabolites and their anthelmintic potential**

Without any exception, all plant species produce different quantities and concentrations of certain elements that possess various biological functions documented to date. These elements have been received plenty of labels (27) such as antinutritional factors, plant secondary compounds, specialized metabolites, and plant secondary metabolites (PSM). The PSM are highly specialized chemical compounds produced by plants through different metabolic pathways derived from the primary metabolism (28). Yet, their presence is influenced by environmental stressful conditions, which has been named plant epigenetic (29). The PSM production is affected by the plant exposition to light, temperature, stress, and specific soil characteristics such as pH and salinity (30,31).

The traditional view in animal and veterinary sciences has tagged the PSM as substances produced with the purpose to avoid herbivory (32). Nonetheless, from an ecological perspective, their production represents specific adaptations improving the resilience, plasticity, and interaction of plants with their environment (33,34). Furthermore, it is necessary to consider that the term herbivore comprises different animal classes such as insects, which have coexisted with PSM for 350 million years (35), or orders such as ruminants, whose families entered into the ecosystem chronology some 18-23 million years (36). Therefore, relationships between animals and PSM are represented by a mutual adaptive response. Consequently, the PSM intake could represent health benefits derived from many mechanisms. These include the bioactive properties against different GIN species (24,25,26). Currently, there are 200,000 PSM (37), albeit this number could represent an under-estimation (38). Because of their heterogeneous chemical composition, many classification systems exist, however, we can divide PSM at follows:

i.) Alkaloids: Which represent the formal beginning of PSM research when the German pharmacist Friedrich Sertürner isolated morphin...
in 1806 (32). This family possess 20,000 structures identified to date (39) and their AH properties have been a current research avenue (40,41).

ii.) Phenolic compounds: Which have 8,000 identified structures (42) and represent the most studied PSM family in relation with the bioactive properties against small ruminants’ GIN. Within this group, the condensed tannins have been received special attention due to their presence in many plants from native vegetation systems worldwide (43,44,45).

iii.) Terpenes: Which represents the largest and highly complex group with some 80,000 identified structures to date (46) and whose AH activity is the aim of the present revision.

Terpenes, terpenoids and isoprenoids

From a structural and chemical perspective, these PSM belong to the most diverse and complex group. The steroids and carotenoids also belong to this PSM family. Currently, a third part of the natural product dictionary ([http://dnp.chemnetbase.com](http://dnp.chemnetbase.com)) belongs to the so-called ‘terpenome’. This PSM group is characterized by a remarkable diversity, whose basic structure follows the principle of isoprene units (C_5)n, also known as 2-methyl-butane. Depending on the sub-units (n) number they are classified as hemiterpenes (C_5), monoterpenes (C_10), sesquiterpenes (C_15), diterpenes (C_20), sesterterpenes (C_25), triterpenes (C_30), and tetraterpenes (C_40) (47). Broadly, terpenes are volatile compounds responsible for the odor in many plant species and fruits. The term “terpene” is derived from a volatile oil (turpentine) obtained from some pine tree species (48). Because their ample distribution within the vegetal kingdom, these PSM could be present in a plethora of botanic families such as Apiaceae, Asteraceae, Euphorbiaceae, Fabaceae, Lamiaceae, Myrtaceae, Poaceae, Rutaceae, Verbenaceae, among many other. In addition, they are synthetized by insects and marine organisms (49). Another important reminder is that, in contrast to terpenes, terpenoids have a structural added group to its chemical composition.

Terpenes are highly lipophilic and volatiles into the environment, constituting a big proportion of the plant organic volatile compounds (OVC). The main constituents of OVC are the monoterpenes and sesquiterpenes, followed by compounds with aromatic rings, fatty acids, and volatile amino acids (50). In contrast, diterpenes and triterpenes are less volatile. OVC are used by fruits and flowers to send signals at some animals, mainly insects (51). In addition, there are reports suggesting that herbivory elicits monoterpenes and some sesquiterpenes in OVC form (48).

The rising interest on terpenes and OVC has triggered the development of methodologies allowing to obtain, isolate, or purify such classes of PSM. These methodologies based — at their majority — on chemical processes have allowed the isolation and characterization of some secondary metabolites. In this sense, the essential oils (EsOi) synthesis represents an alternative to obtain PSM combinations widely used in the animal, pharmaceutical, agricultural, and cosmetic industries, among others.

Essential oils

Define with preciseness the EsOi is not an easy task, given the heterogeneity in their chemical composition and the myriad of properties which have been attributed to them in many human activities such as the pharmaceutical, cosmetic, veterinary, and livestock industries. The table 1 shows some literature concepts about EsOi. Nonetheless, some considerations should be highlighted in order to clarify this concept. Apart from its name, EsOi are not truly oils (lipids). In the same way, the term ‘essential’, does not refer to a like-determinant property, but to their relations with some components responsible for the fragrance or *quinta essentia* in plants (52), thus, its name is derived from the word ‘essence’. EsOi could be obtained from many plant structures (leaves, flowers, stems, fruits, pods, seeds, roots, and barks) or even marine organisms, and represent lipophilic mixtures that contain a variety of chemical compounds (between 20 to 60) obtained by many methodologies (53). Therefore, we might consider the EsOi as oily substances containing different classes of PSM, within which monoterpenes and sesquiterpenes constitute a high proportion. Finally, it is noteworthy to consider that although their bioactive effects could be attributed to their mainly components, synergies between PSM have to be considered and studied.
Table 1. The essential oils concept according to some authors.

<table>
<thead>
<tr>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixtures of secondary metabolites obtained by the plant volatile fraction through steam distillation</td>
<td>(54)</td>
</tr>
<tr>
<td>Highly volatile aromatic compounds extracted from plants by steam or solvent distillation</td>
<td>(55)</td>
</tr>
<tr>
<td>Complex mixtures of bioactive compounds which possess volatile, lipophilic, odoriferous, and liquid substances</td>
<td>(56)</td>
</tr>
<tr>
<td>Volatile and hydrophobic liquids extracted from plants, generally rich in aromas</td>
<td>(57)</td>
</tr>
<tr>
<td>Highly heterogeneous, aromatic, and volatile mixtures of secondary metabolites</td>
<td>(58)</td>
</tr>
</tbody>
</table>

Considering the need for non-pharmacological strategies aimed to face the GIN and the resistance toward commercial therapeutic products, the AH properties of both terpenes (59,60) and EsOi (61) have been documented. Therefore, the review presented here, represents an effort to compile the outcomes of some scientific manuscripts which evaluated the AH activity of terpenes and EsOi in small ruminants under an in vivo approach. In addition, we will discuss their applicability and some knowledge gaps deserving more investigation in the future. We include manuscripts submitted to peer-reviewed journals and published during the last two decades (2001-2021). The inclusion methods were established under the following criteria: (i) manuscripts employing the in vivo approach, (ii) manuscripts focusing on sheep and/or goats, (iii) manuscripts related with a patent GIN infection, (iv) manuscripts using terpene-rich plants or EsOi and, (v) manuscripts associating the intake of terpene-rich plants or EsOi with the GIN infection.

After the search and manuscript selection process, we recovered 11 works fulfilling the inclusion criteria. Table 2 shows some methodological aspects in the analyzed manuscripts. Brazil, with seven works, is the country that leads this research line, followed by three manuscripts performed in the United States of America, and one work executed in the republic of Benin. All manuscripts used the fecal egg count reduction test (FECRT), whereas five employed the controlled test (CnT). The 81% (9/11) of manuscripts used sheep and only the 19% (2/11) used goats. The mean (± standard deviation) of experimental animals was 27.6 ± 12.3 per work. The 64% (7/11) of manuscripts involved a natural GIN infection, whereas the 36% (3/11) opted for an artificial infection, at its majority with the abomasal nematode Haemonchus contortus, and on one opportunity, a mixed infection with H. contortus and the intestinal nematode Trichostrongylus colubriformis.

Table 3 shows the plant species and chemical compounds involved in the analyzed works. Although we cannot identify a predominant botanical family, both Lamiaceae (n = 2) and Rutaceae (n = 2) accounted for the 36% of works (4/11). The use of a terpene-rich plant foliage was documented for two of the eleven works (18%). Consequently, the EsOi administration occupied the majority of works. Altogether, the analyzed works focused on EsOi, studied 76 PSM, among which were reported monoterpenes, sesquiterpenes, phenyl propanoids, hydrocarbons, acids, alcohols, aldehydes, phenols, acyclic esters, and lactones among others.

The outcomes dealing with the intake of terpenes and/or EsOi and GIN infection are presented in table 4. Broadly speaking, the AH activity of terpenes and EsOi was variable, with fecal egg excretion reduction values between 0 to 97.4%, according with the FECRT. The CnT approach was utilized in five of the eleven works (45%) and shows variable outcomes.

The in vivo approach on the evaluation of compounds with bioactive potential in small ruminants

The review presented here aimed to analyze some scientific manuscripts that evaluated the AH activity of terpenes and EsOi in small ruminants through the execution of in vivo trials. This approach, which directly involves the animal, has been an important component in the evaluation of synthetic and natural products within veterinary sciences. However, it is worthy to highlight that an official guideline for natural product evaluation in ruminants has not yet been published. For this reason, it is reasonable to emphasize in the current need for official documents endorsed by scientific committees. We, as part of the veterinary parasitologists community, are waiting for official organisms such as the World Association for the Advancement of Veterinary Parasitology (WAAVP) or the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) publication of such guidelines in the near future.
Table 2. General information of manuscripts aimed to evaluate the anthelmintic activity of terpenes and essential oils through the *in vivo* approach in small ruminants.

<table>
<thead>
<tr>
<th>Country</th>
<th>Test type</th>
<th>Species</th>
<th>Breed</th>
<th>n</th>
<th>Age</th>
<th>Experimental condition</th>
<th>Infection type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>FECRT CnT</td>
<td>Goat</td>
<td>Boer, Alpine and Nubian</td>
<td>19</td>
<td>0-5 y</td>
<td>Pen</td>
<td>Natural MS, Hc</td>
<td>(62)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>1-3.5 m</td>
<td>4-5 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>FECRT CnT</td>
<td>Sheep</td>
<td>Hair</td>
<td>44</td>
<td>NS</td>
<td>Pen</td>
<td>Natural</td>
<td>(63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>FECRT</td>
<td>Goat</td>
<td>NS</td>
<td>30</td>
<td>12-16 m</td>
<td>Field</td>
<td>Natural + MS, Hc</td>
<td>(64)</td>
</tr>
<tr>
<td>USA</td>
<td>FECRT</td>
<td>Sheep</td>
<td>Mixed</td>
<td>18</td>
<td>3 m</td>
<td>Pen</td>
<td>MS, Hc</td>
<td>(65)</td>
</tr>
<tr>
<td>Brasil</td>
<td>FECRT CnT</td>
<td>Sheep</td>
<td>Santa Inés</td>
<td>18</td>
<td>2 m</td>
<td>Pen</td>
<td>MS, Hc</td>
<td>(66)</td>
</tr>
<tr>
<td>USA</td>
<td>FECRT</td>
<td>Sheep</td>
<td>Blackbelly, Saint Croix</td>
<td>64</td>
<td>6 m</td>
<td>Pen</td>
<td>Natural</td>
<td>(67)</td>
</tr>
<tr>
<td>Brazil</td>
<td>FECRT</td>
<td>Sheep</td>
<td>NS</td>
<td>30</td>
<td>6-16 m</td>
<td>Pen</td>
<td>Natural</td>
<td>(68)</td>
</tr>
<tr>
<td>Brazil</td>
<td>FECRT</td>
<td>Sheep</td>
<td>Santa Inés</td>
<td>30</td>
<td>4-6 m</td>
<td>Pen</td>
<td>MS, Hc</td>
<td>(69)</td>
</tr>
<tr>
<td>Benin</td>
<td>CnT</td>
<td>Sheep</td>
<td>Djallonke</td>
<td>15</td>
<td>4-6 m</td>
<td>Field</td>
<td>Natural Mix, Hc + Tc</td>
<td>(70)</td>
</tr>
<tr>
<td>Brazil</td>
<td>FECRT</td>
<td>Sheep</td>
<td>Santa Inés</td>
<td>28</td>
<td>7 m</td>
<td>Pen</td>
<td>Natural</td>
<td>(71)</td>
</tr>
<tr>
<td>Brazil</td>
<td>FECRT CnT</td>
<td>Sheep</td>
<td>Santa Inés, Morada Nova</td>
<td>32</td>
<td>5 m</td>
<td>3 m</td>
<td>Pen</td>
<td>MS, Hc Natural</td>
</tr>
</tbody>
</table>

Country: USA United States of America; Test type: FECRT Fecal egg count reduction test, CnT controlled test; n number of experimental animals; Age: y years, m months; Infection Type: MS Monospecific, HC *Haemonchus contortus*, Tc *Trichostrongylus colubriformis*

Table 3. Plant species, administration mode, and chemical composition of tested material in the analyzed manuscripts.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Family</th>
<th>Presentation</th>
<th>Chemical compounds</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chenopodium ambrosioides</td>
<td>Epazote, paico</td>
<td>Amaranthaceae</td>
<td>Leaves, Essential oil</td>
<td>[4]: Ascaridole, p-cymene, α-terpinene, limonene</td>
<td>(62)</td>
</tr>
<tr>
<td>Lippia sidoides</td>
<td>Pimienta de romero</td>
<td>Verbenaceae</td>
<td>Essential oil</td>
<td>NS</td>
<td>(63)</td>
</tr>
<tr>
<td>Eucalyptus staigeriana</td>
<td>Eucalipto</td>
<td>Myrtaceae</td>
<td>Essential oil</td>
<td>[20]: α-pinene, β-pinene, β-myrcene, α-phellandrene, O-cymene, (+)-limonene, eucaliptol, y-terpinene, α-terpinolene, β-linalool, β-citronellal, 4-terpineol, α-terpineol, cis-geraniol, Z-citral, trans-geraniol, E-citral, metil geranate, nerol acetate, and geraniol acetate</td>
<td>(64)</td>
</tr>
<tr>
<td>Citrus sinensis</td>
<td>Naranja valenciana</td>
<td>Rutaceae</td>
<td>Essential oil</td>
<td>d-limonene represented the 95% of solution</td>
<td>(65)</td>
</tr>
<tr>
<td>Cymbopogon schoenaustus</td>
<td>Zacate limón, limoncillo</td>
<td>Poaceae</td>
<td>Essential oil</td>
<td>[19]: Geranial, geranial, nerol, geraniol acetate, citronelol, (E) carophyllene, linalool, citronelol, (E)-2-hexenal, γ-cadinene, canephellandrene oxid, N-decanal, α-Metil-5-heptene-2-one, eugenol, citronelil acetate, α-humulene, cadine 1,4 diene, geranyl formate, α-murolene</td>
<td>(66)</td>
</tr>
<tr>
<td>Juniperus pinchotii</td>
<td>Enebro de mora roja</td>
<td>Cupressaceae</td>
<td>Leaves</td>
<td>The plant contained ‘good’ concentrations of condensed tannins and terpenoids</td>
<td>(67)</td>
</tr>
<tr>
<td>Thymus vulgaris</td>
<td>Tomillo</td>
<td>Lamiaceae</td>
<td>Essential oil</td>
<td>[13]: Thymol, p-cymene, γ-terpinene, linalool, β-duprezaínone, α-pinene, camphor, sabinene cis-hidrate, isoborneol, trans-β-terpineol, myrjene, α-fenchane, (2)-tagetone</td>
<td>(69)</td>
</tr>
<tr>
<td>Zanthoxylum zanthoxyloides</td>
<td>Fagara ‡</td>
<td>Rutaceae</td>
<td>Essential oil</td>
<td>[27]: γ-terpinene, undecane, valencene, decanal, 3-carene²</td>
<td>(70)</td>
</tr>
<tr>
<td>Mentha arvensis</td>
<td>Menta Japonesa</td>
<td>Lamiaceae</td>
<td>Essential oil</td>
<td>Menthol represented the 86.7% of essential oil³</td>
<td>(71)</td>
</tr>
</tbody>
</table>

‡ Common name in African countries; NS: Not Specified; ¹ A 40% emulsión with 40% Orange oil was administered; Chemical Compounds: The number between Brackets represent the number of secondary metabolites detected in the essential oil; ² Reported 25 secondary metabolites, but those put in the table are the only reported; ³ Do not reported more secondary metabolites; (68) and (72) were excluded from this table because they used commercial solutions of monoterpenes
Table 4. Bioactive effect of plants and essential oils on gastrointestinal nematodes of small ruminants through the in vivo approach.

<table>
<thead>
<tr>
<th>Test type</th>
<th>Material</th>
<th>Presentation</th>
<th>Dose</th>
<th>Results FECRT (↓ epg)</th>
<th>Results CnT</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FECRT</td>
<td>Chenopodium ambrosioides</td>
<td>Leaves, Essential oil</td>
<td>0.2 mL/Kg, 250 g</td>
<td>NS</td>
<td>NS</td>
<td>(62)</td>
</tr>
<tr>
<td>CnT</td>
<td></td>
<td></td>
<td>NR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FECRT</td>
<td>Lippia sidoides</td>
<td>Essential oil</td>
<td>230-283 mg/kg</td>
<td>22.9 - 54.22%</td>
<td>Hc</td>
<td>(63)</td>
</tr>
<tr>
<td>CnT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>represented the 37.9% of infection whilst Tc represented the 62.1%</td>
<td></td>
</tr>
<tr>
<td>FECRT</td>
<td>Eucalyptus staigeriana</td>
<td>Essential oil</td>
<td>350 mg/kg</td>
<td>73.66%</td>
<td>†</td>
<td>(64)</td>
</tr>
<tr>
<td>FECRT</td>
<td>Citrus sinensis</td>
<td>Essential oil</td>
<td>600 mg/kg1</td>
<td>94.9 – 97.4%</td>
<td>†</td>
<td>(65)</td>
</tr>
<tr>
<td>FECRT</td>
<td>Cymbopogon schoenanthus</td>
<td>Essential oil</td>
<td>180-360 mg/kg</td>
<td>NS</td>
<td>NS</td>
<td>(66)</td>
</tr>
<tr>
<td>CnT</td>
<td>Juniperus pinchotii</td>
<td>Leaves, 30% inclusion</td>
<td>65%</td>
<td></td>
<td>†</td>
<td>(67)</td>
</tr>
<tr>
<td>FECRT</td>
<td>Carvaryl acetate</td>
<td>Essential oil</td>
<td>250 mg/kg</td>
<td>65.9%</td>
<td>†</td>
<td>(68)</td>
</tr>
<tr>
<td>FECRT</td>
<td>Thymus vulgaris</td>
<td>Essential oil</td>
<td>75-150-300 mg/kg</td>
<td>NS</td>
<td>†</td>
<td>(69)</td>
</tr>
<tr>
<td>CnT</td>
<td>Zanthoxylum zanthoxyloides</td>
<td>Essential oil</td>
<td>1-2 mL/Kg2</td>
<td>89%</td>
<td>The number of adult GIN was reduced. Female prolificity was also reduced</td>
<td>(70)</td>
</tr>
<tr>
<td>FECRT</td>
<td>Mentha arvensis</td>
<td>Essential oil</td>
<td>160-200 mg/kg</td>
<td>44.9 – 61.6% (NS)</td>
<td>†</td>
<td>(71)</td>
</tr>
<tr>
<td>FECRT</td>
<td>Encapsulated anethol and</td>
<td>Essential oil</td>
<td>100-250 mg/kg*</td>
<td>55% at 47 days</td>
<td>Fertility of adult GIN was reduced</td>
<td>(72)</td>
</tr>
</tbody>
</table>

Test type: FECRT Fecal egg count reduction test, CnT Controlled test; Dose: mL milliliter, Kg kilogram, NS Not specified, mg milligrams; 1 Used peel orange essential oil; 2 Used essential oil from Zanthoxylum zanthoxyloides fruits; Results: ↓ epg reduction in excretion of gastrointestinal nematode eggs, NS not significative, † does not apply; Hc Haemonchus contortus, Tc Trichostrongylus colubriformis, GIN Gastrointestinal nematodes

As a consequence, the works performed to date have utilized the official guidelines to assess the synthetic compounds effectivity (73) or some published data from field experts (74). These methodological strategies involve the study of plants / extracts AH properties over distinct stages of the GIN biological cycle (eggs, larvae, and adults) at the laboratory level, constituting the in vitro approach.

In vitro trials are cheaper and bring the possibility to examine a relatively higher number of samples in a shorter time period (75), thereby have been more used and documented. Based on our literature search, we can state that for every seven to ten manuscripts focusing on the in vitro approach, there is one manuscript dealing with the in vivo approach. However, in vitro trials do not face the multifactorial physiological conditions of animals in which: (i) there is a dynamic between quantities and concentrations of chemicals who enter into the system, (ii) the rate and sequence in which PSM are processed is dynamic and variable, (iii) high intakes of fresh material would be necessary to reach the concentrations that showed positive results on in vitro conditions, and (iv) rumen microbial populations have the capacity to develop adaptations toward PSM. Therefore, own to the difficulty to duplicate these factors, evaluate on the host represents the best choice to assess the AH value of plants and their derivatives (75).

Two methodologies assessing the AH activity of natural products are usually implemented on the in vivo approach (72):

Fecal egg count reduction test (FECRT); in which the bioactive material is administered to a group of animals and a quantitative follow-up of the number of GIN eggs per gram (EPG) in feces is estimated by establishing a time period (pre
and pos). In this approach, the establishment of a control group is optional. Thus, researchers can define whether other experimental groups do not receive the bioactive material or are free of GIN infection.

Controlled test (CnT); which is similar to FECRT, but the establishment of control groups is mandatory because experimental animals should be humanely euthanized to retrieve organs of the gastrointestinal tract where adult nematodes are located. After this step, counts and body measurements of adult GIN individuals are performed, which allows for comparison between experimental groups. Although this methodology is expensive and labor consuming, is the most reliable to evaluate the AH activity of natural products.

**Experiences in the utilization of terpenes and essential oils over the small ruminants’ gastrointestinal nematodes**

The FECRT was implemented in all the analyzed manuscripts in the present review. The outcomes in EPG dynamics after administration of terpenes or EsOi were certainly ambiguous, showing activities considered as null (62,66,69), moderate (63,67,68), positive (64,70) and remarkable (63). Although these responses are not conclusive and were derived from a limited number of works, they allow us to detect a potential in the administration of terpene-rich resources with applicability on small ruminants. In the near future, it would be interesting to explore whether other factors could influence the bioactive properties of this natural products such as dose, bioavailability, plant harvest season, extraction method for terpene/EsOi obtention, and interaction between PSM. In the same way, factors such as administration route, productive performance, nutritional plane, physiological stage, and animal PSM adaptation level should be accounted for.

In the present review, five studies implemented the CnT approach to evaluate the AH activity of terpene-rich plants or EsOi and, similarly with the FECRT, the outcomes were variable. In the works of Ketzis et al (62) and Katiki et al (66), the administration of *Chenopodium ambrosioides* and *Cymbopogon schoenanthus* EsOi, respectively had no effects on the adult GIN counts recovered after necropsy. Noteworthy outcomes were reported in Camurca et al (63), who observed certain selectivity of the *Lippia sidoides* EsOi over *Haemonchus contortus* populations. Also, the EsOIs of *Zanthoxylum zanthoxyloides* fruits (70) and an encapsulated solution of the monoterpenes anethol and carvone (72) reduced the number of adult GIN, male size, and number of produced eggs in females.

The mechanisms used by terpenes and EsOi to negatively impair the GIN, are still unknown. However, it has been proposed that due to their hydrophobic nature, the cell membrane is the main target (76). These compounds enter GIN structures through transcuticular diffusion interfering the metabolism, avoiding vital functions on early developmental stages, and affecting some locomotion mechanisms (77). According with López and Pascual-Villalobos (78), EsOi show activity toward acetylcholinesterase receptors of vertebrates and invertebrates, producing a neurotoxic damage similar to organophosphates. Notwithstanding, is necessary to bear in mind that terpene / EsOi bioactivity might be as ample as their high number of identified molecules.

Research and development of natural AH products has been triggered because the growing populations of GIN resistant to commercial products. In this revision, ivermectin resistance on GIN populations was evident, by reporting EPG reductions of 67-85% (63), 35-54% (62), and 25% (67). In addition, some studies used the most recently introduced AH into the market (monepantel, Zolvix®) evidencing reduction levels of 100% (69), 96.4% (68) and, albeit the figures presented by Katiki et al (72) lacks a specific date, we can observe —clarifying that it requires a confirmation— some resistance level after 21 days of administration. These parasitological variables could be complemented with the assessment of physiological variables such as the packed cell volume (known as hematocrit), FAMACHA®, white cells count (66,67,70,72), and renal / hepatic function assessment (66,72). Additionally, the follow-up of productive variables such as the daily weight gain and feed conversion are of great importance given their applicability on different productive systems. In this sense, Whitney et al. (67) reported a reduction in the weight gain of Blackbelly and Saint Croix lambs after the inclusion of 30% *Juniperus pinchotii* in a balanced diet. These results were similar to those of Katiki et al (72) who reported a reduction in the daily weight gain on Santa Inés lambs. By contrast, Azando et al (70) reported that administration of EsOi from the fruit of *Z. zanthoxyllum* did not
affected neither the daily weight gain nor the final live weight of Djallonke lambs. Despite the fact that results suggest a negative trend over productive variables, future works should establish the *trade-off* costs between nutrition and health (79,80).

Terpene and EsOi possess a wide spectrum of biological activities that could be explored in veterinary sciences. A recent global review about terpenes in animals reported that 58% of 113 analyzed manuscripts were conducted on livestock species destined to human consumption (81). Currently, 80,000 plant species have been identified for their influence on animal health and productivity (82), therefore, it is necessary to understand the nature of their interactions with herbivores, and thus, suggest their nutritional and pharmaceutical properties in different small ruminant productive systems (83,84). These benefits could be characterized on functional foods or nutraceutical resources.

**Interest conflict**

The authors of the present study declare that any interest conflict exists with the publication of this manuscript.

**REFERENCES**


