UNIVERSIDAD DE SANTIAGO DE CHILE

BOLETÍN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
18 (6): 533 - 543 (2019)
© / ISSN 0717 7917 / www.blacpma.usach.cl

Blacpma

Revisión | Review

Potential of botanical driven essential oils against *Haemonchus contortus* in small ruminants

[Potencial de aceites esenciales botánicos contra *Haemonchus contortus* en pequeños rumiantes]

Muhammad Rashid Fayaz[1], Rao Zahid Abbas[1], Asghar Abbas[2], Muhammad Kasib Khan[1], Muhammad Asif Raza[2], Muhammad Israr[3], Junaid Ali Khan[4], Muhammad Shahid Mahmood[5], Muhammad Kashif Saleemi[6], Tauseef ur Rehman[7], Muhammad Arfan Zaman[8] & Zia ud Din Sindhu[1]

[1]*Department of Parasitology, University of Agriculture Faisalabad, Pakistan*
[2]*Department of Veterinary and Animal Sciences, Muhammad Nawaz Shareef University of Agriculture Multan, Pakistan*
[3]*Pakistan Science Foundation, Islamabad-Pakistan*
[4]*Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture Faisalabad, Pakistan*
[5]*Institute of Microbiology, University of Agriculture Faisalabad, Pakistan*
[6]*Department of Pathology, University of Agriculture Faisalabad, Pakistan*
[7]*University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur-Pakistan*
[8]*University College of Veterinary and Animal Sciences, Jhang-Sub Campus, The University of Veterinary and Animal Sciences, Lahore-Pakistan*
Contactos | Contacts: **Rao Zahid ABBAS** - ***E-mail address:*** **raouaf@hotmail.com**

Abstract: The livestock sector is continuously facing problems in controlling parasitic diseases especially Haemonchosis due to emergence of anthelminthic resistance and failure in vaccination control programmes. Therefore, to increase milk and meat production and emerging demand of meat free from drug residues development of new alternative approaches are appealing for prevention and control of Haemonchosis in small ruminants. Among alternatives, plants driven essentials oils have shown promising results in control of *Haemonchus contortus* infection at various concentrations by different assays including egg hatch assay, larval development assay, larval exsheathment assay and adult motility assay. Essential oils are complex mixtures of various impulsive or volatile compounds which have potential to control Haemonchosis. The current study reviews the therapeutic effects of essential oils of plants against *Haemonchus contortus* and to be used them against *Haemonchus contortus* for future perspectives.

Keywords: Essential oils; *Hemaonchus contortus*; Resistance; Parasites.

Resumen: El sector ganadero enfrenta continuamente problemas para controlar las enfermedades parasitarias, especialmente la hemoncosis, debido a la aparición de resistencia antihelmíntica y al fracaso en los programas de control de vacunación. Por lo tanto, para aumentar la producción de leche y carne, y la demanda emergente de carne libre de residuos de medicamentos, el desarrollo de nuevos enfoques alternativos es atractivo para la prevención y el control de la hemoncosis en pequeños rumiantes. Entre las alternativas, los aceites esenciales producidos por las plantas han mostrado resultados prometedores en el control de la infección por Haemonchus contortus a diversas concentraciones mediante diferentes ensayos, incluido el análisis de eclosión de huevos, el desarrollo de larvas, el análisis de vaciado de larvas y el ensayo de motilidad en adultos. Los aceites esenciales son mezclas complejas de varios compuestos impulsivos o volátiles que tienen potencial para controlar la hemonchosis. Este estudio revisa los efectos terapéuticos de los aceites esenciales de las plantas contra Haemonchus contortus y evalúa sus perspectivas futuras como agentes para combatir las enfermedades causadas por este parásito.

Palabras clave: Aceites esenciales; *Hemaonchus contortus*; Resistencia; Parasitos.

Recibido | Received: March 19, 2019
Aceptado | Accepted: August 23, 2019
Aceptado en versión corregida | Accepted in revised form: August 28, 2019
Publicado en línea | Published online: November 30, 2019
Este artículo puede ser citado como / This article must be cited as: MR Fayaz, RZ Abbas, A Abbas, MK Khan, MA Raza, M Israr, JA Khan, MS Mahmood, MK Saleemi, T Rehman, MA Zaman, ZD Sindhu. 2019. Potential of botanical driven essential oils against *Haemochus contortus* in small ruminants. **Bol Latinoam Caribe Plant Med Aromat** 18 (6): 533 – 543. **Https://doi.org/10.35588/blacpma.19.18.6.36**

INTRODUCTION

All over the world livestock population has become susceptible to parasitic diseases affecting their health as well production performance (Mehmood *et al*., 2017; Khater *et al*., 2018; Kiran *et al.,* 2018). In livestock gastrointestinal parasites having vital socio-economic importance lead to clinical as well subclinical infections. The gastrointestinal burden of parasites includes *Haemonchus, Oesophagostomum, Ostertagia, Cchabertia, Nematodirus, Trichuris, Fasciola* and *Moniezi*. *Haemonchus* belongs to kingdom Animalia, Phylum Nematode, Family *Tricostrongylidae*. The subfamily *Haemonchoidae* consists of four species in domestic animals including *Haemonchus contortus* (Caprine and ovine), *Haemonchus similis*, *Haemonchus placei* (Bovines) and *Haemonchus longistipes* (Camels) (Inaam *et al.,* 2007). *Haemonchosis* is the major threat to small ruminants in terms of health, productive efficiency and economic point of view. *Haemonchus contortus* remains in abomasum and sucks blood hence called blood sucking parasite in small ruminants (Ijaz *et al.,* 2009). The clinical pathogenesis of *Haemonchosis* include loss of appetite, weakness, dehydration, rough skin appearance, pale mucus membrane, debilitating condition, decreased milk production, and chronic wasting and severe anemia due to blood loss ultimately leading to death of animal (Rodríguez *et al.,* 2015). In *Haemonchosis* each parasite sucks blood 0.05ml per day and ultimately anorexia, anemia and chronicity of infection lead to death of host. The 10000 worms of *Haemonchus* are sufficient to kill the goat or sheep (Burke *et al.,* 2004).

Traditionally various control strategies are being attempted to reduce *Haemonchosis* risk in small ruminants (sheep and goats). *Haemochus contortus* have been usually controlled by chemotherapeutic agents like anthelminthic, vaccination and also by rotational grazing management in livestock population. The chemotherapeutic agents showed satisfactory results to control *Haemonchus contortus* in sheep and goats. However, blindly use of anthelmintic drugs has shown resistance against *Haemonchus contortus*. Frequent use of anthelmintic drugs has shown resistance against *Haemonchus contortus* (Besier & Love, 2003). The first drug shown resistance against *Haemochus contortus* was phenothiazine in sheep. Later on Thiabendazole was documented to be resistant for nematodes in livestock (Drudge *et al.,* 1964). The *Haemonchus contortus* have been documented to be resistant against various classes of anthelminthic like Benzimidazole, Imidazothazole, Ivermectin and Salicylanilides (Katiki *et al.,* 2012). Anthelmintic resistance in gastrointestinal nematodes is a worldwide problem. Multi-drug resistant *Haemonchosis* is the most serious impediment for small ruminant systems, and there are no new drug candidates currently under development (Araújo *et al.,* 2017; Katiki *et al.,* 2017; Ijaz *et al.,* 2018).

Vaccination is one of the authentic method to control the parasitic infestation in livestock population (Lejambre *et al.,* 2008). The major hindrance in commercialization of *Haemonchus contortus* vaccine is the manufacturing of recombinant proteins that move toward the effectiveness of better native antigens. The vaccine provides round about 65% protection against *Haemonchosis* that is not satisfactory (Besier *et al.,* 2015). Moreover the vaccine schedule that is being applied now a days is unprofitable. However it is not clear about its usage in neither ruminants nor its advantage as well withdrawal period.

Due to synthetic drug resistance and vaccination failure, there is need to be used alternate technologies to control parasitic problem in livestock population (Khan *et al.,* 2017; Mahmood *et al.,* 2018; Soares *et al.,* 2018). Among alternatives, botanicals and their essential oils have shown favorable effects against external and internal parasites (Katiki *et al.,* 2012; Katiki *et al.,* 2017; Abbas *et al*., 2017a; Abbas *et al*., 2017b; Idris *et al.,* 2017; Khan *et al.,* 2018). Essential oils, are complex mixtures of impulsive or volatile compounds which are particularly abundant in aromatic plants and have diverse therapeutic properties against parasitic, and bacterial diseases. These compounds are mainly composed of terpenes which are biogenerated by mevalonate pathway. These volatile molecules include monoterpenes (oxygenated monoterpens, hydrocarbon), and also sesquiterpenes (hydrocarbon and oxygenated sesquiterpens) (Dhifi *et al.,* 2016).

In this review importance of essential oils, their origin, their role in controlling *Hemaonchus contours* including their mechanism of action and limitations are comprehensively discussed below.

Table No. 1
Status of Anthelmentic drug resistance to *Haemonchus contortus* in different regions of the world

Anthelmentic Drugs (Class)	Country	Reference
Imidazothiazoles class (Levamisole)	Australia	Sangster, 1999
	New Zealand	Gopal *et al.*, 1999
	Kenya	Rolfe *et al.*, 1990
	Pakistan	Khan *et al.*, 2017
Salicylanilides (Closantel)	Australia	Sangster, 1999
	Kenya	Rolfe *et al.*, 1990
	Canada	Prichard, 1994
Macrolitic Lactones (Avermectins & moxidectin)	Kenya	Waruiru *et al.*, 1998
	Australia	Sangster & Gill, 1999
	Pakistan	Khan *et al.*, 2017
	Indonesia	Puspitasari *et al*., 2016
Benzimedazoles	Brazil	Uppal *et al.*, 1992
	Pakistan	Khan *et al.*, 2017
	Malaysia	Rahman, 1994
	India	Uppal *et al.*, 1992
	kenya	Mwamachi *et al*., 1995
	Brazil	Echevarria *et al.*, 1991
	New Zealand	Gopal *et al.*, 1999
	Australia	Sangster & Gill, 1999
	Indonesia	Puspitasari *et al.*, 2016
	Malysia	Sivaraj *et al.*, 1994

Anthelmintic effect of Different botanical driven Essential Oils against Haemonchus contortus

Hesperozygis myrtoids

Essential oil of *Hesperozygis myrtoides* (A.St.-Hil. ex Benth.) used against *Haemonchus contortus* given a new path toward ethno-veterinary products to use for gastrointestinal parasites. *Hesperozygis myrtoides* commonly called as *"poejo" (pen-nyroyal)* have perfect aroma which related to essential oil that include monoterpene ketones. To control *Haemonchus contortus*, egg hatching and larval development tests were performed in concentrations of 0.012 mg/ml to 25 mg/ml and 0.003 mg/ml to 0.4 mg/ml respectively. The main components identified in the essential oil were isomenthone (47.7%), limonene (7.7%), pulegone (21.4%), isomenthyl acetate (6.8%) and neoisomenthol (3.9%). The essential oils of *H. myrtoides* showed the anthelmintic effectiveness against *Haemonchus contortus* (Castilho *et al.,* 2017).

Artemisia species

Plants of genus *Artemisia* include various plants which are used against diseases like hepatitis, malaria, cancer (Rustaiyan & Masoudi, 2011) and also used as anthelmintics. The essential oil of *Artemisia* act as synergistically within and other constituents of essential oils way to target the parasitic stages by various mechanism (Marie-Magdeleine *et al.,* 2009). *In vitro* study of essential oil of *Artemisia lancea* (Vaniot) first time studied against *Haemonchus contortus* by larval migration inhibition assay were camphor 16.65% and 1,8-cineole 34.56 %. The major components of essential oil were 1,8-cineole (34.56%) and camphor (16.65%). Hence the activity of essential oil of the *Artemisia launcea* proved to be effective against various stages of *Haemonchus contortus* (Zhu *et al.,* 2013a).

Eucalyptus species

Eucalyptus belonging to Myrtaceae family known to have as acaricidal (Chagas *et al*., 2002), insecticidal (Maciel *et al*., 2010) and nematicidal effects (Macedo *et al*., 2010). Myrtaceaefamily local to Australia grown majorly for cosmetic, pharmaceutical and paper industry (Hasegawa *et al*., 2008). *In vitro* anthelmintic activity of essential oils of *Eucalyptus staigeriana* (F.Muell. ex F.M.Bailey) performed on larvae and eggs of *Haemonchus contortus*. *Eucalyptus staigeriana* essential oil arrested the larval hatchability 99.96 % at concentration of 1.0 mg/ml. The major chemical constituents of essential oil were Limonene (72.9%), 1,8-Cineole (9.5%) and o-Cimene (4.6%) (Ribeiro *et al.,* 2013).

In another study, essential oil of *Eucalyptus citriodorais* proved to be effective in prohibition of *H. contortus* larvae development (Macedo *et al.,* 2011).

Thymus vulgaris

Thymus vulgaris (Reut. ex Willk.) essential oil consists of monoterpene thy-mol and carvacrol which are isomers of thy-mol (Walentowska & Foksowicz-Flaczyk, 2013). The *Thymus vulgaris* used in different purpose like as anthelminthic as well toxic tolerability effects in mammals (Behnia *et al.,* 2008). *Thymus vulgaris* proved to be pharmacological effects in food supplement medicines (Behnia *et al.,* 2008). The essential oil of *Thymus vulgaris* has three possible mode of actions like arrest larval development, motility and hatchability by active component. Thymol is major components of essential oil which accounts for 50.22% of total oil composition. The Thymol oil prohibited the egg hatchability of *Haemonchus contortus* up to 96.4 to 100 %, larval motility 97 to 100 % and 90 to 100 % larval development in an *in vitro* study (Ferreira *et al.,* 2016).

Arisaema spp

Arisaema franchetianum (Engler) and *Arisaema lobatum* (Engler) are two perennial plants native to China (Zhao *et al.,* 2010). It is being thought that essential oils of *Arisaema franchetianum* and *Arisaema lobatum* are secondary metabolites of volatile medial plants used for wide activities like antioxidant, antibacterial, analgesic, anti-inflammatory and anthelminthic biological programs (Adorjan & Buchbauer, 2010; Shaaban *et al*., 2012). In one study, *Arisaema franchetianum* and *Arisaema lobatum* essential oils showed more than 99 percent egg hatch inhibition of *Haemonchus contortus* at concentration of 10 mg/ml and 5-10 mg/ml respectively (Zhu *et al.,* 2013b). Carvacrol and linalool were the major constituents of both tested essential oils.

Skimmia laureola

Essential oils from *Skimmia laureola* (Siebold & Zucc. ex Walp) leaf, stem and root were tested for their anthelmintic activity against *Haemochus contortus* by "Adult motility assay". It was found that leaf, stem and root of *S. laureola* essential oil have anthelminthic effects. Essential oils of *S. laureola* stem and root has ruined the parasite by altering body color by inhibiting the circulatory system or skin destruction. β-linalool and 1,3-cycloheptadiene were major components of tested essential oils. Hence, *S. laureola* having potential to damage the *Haemonchus contortus* by acting on membrane (Mehmood *et al.*, 2011).

Ruta chalepensis

Ruta chalepensis (Carl Linnaeus) essential oil having ability as anthelmintic and insecticidal and different studies proved that flowers and leaves of *Ruta chalepensis* showed valuable potential as insecticidal as well anthelmintic. Leaves essential oils showed higher prohibitory effect on the egg hatching ability than flowers. Moreover it declared that at various concentrations *Ruta* genus was tested to evaluate the activity of essential oil obtained from one major portion of this plant is being used worldwide (Verzera *et al.*, 2000). *Ruta chalepensis* leaves and flowers essential oil at concentration of 1 mg/ml proved to be reduced the 33.3% *Haemonchus contortus* in egg hatching assay test (Akkari *et al.*, 2015). Essential oil mostly contained ketone (2-undecanone derivative) in considerable amounts (85%).

Melaleuca alternifolia and Terpinen -4-ol

The *in vitro* activity of *Melaleuca alternifolia* and terpinen -4-ol were tested on larvae and eggs of *Haemochus contortus* parasite. Inhibition of larval migration test and egg hatching inhibition were used to know the effectiveness. It was concluded that *Melaleuca alternifolia* has 100% effectiveness against *Haemochus contortus* and terpinen-4-ol have 82 percent at same concentration. So, it was proved that these plant constituents act as larvicidal as well ovicidal effects against *Haemonchus contortus* (Grando *et al.*, 2016).

Mode of action of Essential Oils

The mode of action of essential oils are described as it prohibits the growth of enzymes like Adenosine triphosphatases (ATPases) and acetylcholinesterases (AChE) that requires for the growth and energy relationship of worms and inhibit the GABA receptor of parasite (Zahran *et al.*, 2017). Some essential oils have shown to be ascaricidal and anthelminthic that rich in methone, isomethane, and pulegone enriched monoterpeneketones (Chagas *et al.*, 2014). Normally essential oils showed to inactivate the structures of parasites. They also act to be disrupting the cell membranes by changing permeability of lipophilic compounds leading release of various enzymes as well disturb the nutrients (Cox *et al.*, 2000).

Essential oils act directly or indirectly on various parasites by modifying the host immune response or act on parasite by providing safety to the host (Machado *et al.*, 2010). Moreover it is considered that essential oils cause breakdown of the proteins and releases free amino acids that ultimately influence protein level in the cell (Upadhyay, 2010). Essential oils have also shown to inhibit the various physiological processes like biochemical arresting the nutrition change in structure effect neuromuscular transmitters of helminths. These are the popular functions to inhibit the helminths in various ways (Mehmood *et al.*, 2011).

Restrictions with Essential Oils

The increasing demand and pressure on the livestock industry has initiated new research to find safe, cheap and efficient alternatives. No doubt essential oil having wide potential to use and are safe to use but many studies conducted shown toxic effects of these. However, the applications of these compounds are limited due to questions regarding their potent undesirable aspects such as carcinogenicity, acute toxicity, teratogenicity and slow degradation periods (Calo *et al.*, 2015). The essential oils also potentiate cytotoxic effects in animals that are treated (Christaki *et al.*, 2004). Essential oils led to deteriorates the cell membrane and cell wall structure (Bakkali *et al.*, 2008). Essential oils effect the depolarization of mitochondrial membrane in cell by altering ion channels and reduce ATP production (Vercesi *et al.*, 1997). Likewise, thymol and carvacol essential constituents of various essential oils mentioned to be lethal for the intestinal cells of mucosa layer due to lipophilic and hydrophobic(Giannenas *et al.*, 2003).

Use of essential oils is under discussion to be using them as potential candidate against gastrointestinal parasites in animals. Hence there is

need to study the essential constituents, their lethal or toxic effect and mechanism of action in controlling Haemochus contortus (Athanasiadou *et al*., 2007).

Table No. 2
In vitro* efficacy of different botanical driven essential oils against *Haemonchus contortus

Plant name	Dose	Composition	EHA	LMA	LDA	Reference
Mentha piperita	0.26 mg/ml	Menthol (42.5%)	95 %	-	95%	Katiki *et al.*, 2011
Cymbopogon martinii	0.15 mg/ml	Geraniol (81.4%)	95%	-	95%	
Cymbopogon schoenanthus	0.15 mg/ml	Geraniol (62.5%)				
Newbouldia laevis	18.2 µg/ml	B-caryophyllene (36 %) & Eugenol (5.8 %).		95%		Olounlade *et al.*, 2012
Zanthoxylum zanthoxyloides	19.5 µg/ml	γ-terpinene (18 %) & undecane (15 %	98%	95%		
Ocimum grastissimum	46 µg/ml	Eugenol (55.6%)	100%	15%		Pessoa *et al.*, 2002
Allicin (*garlic*)	30 ug/ml	Diallyl disulfide (27.9%)& Diallyl trisulfide (33%)		15%		Ankri & Mirelman, 1999
Artemisia absinthium	20 %					Zhu *et al.*, 2013a; Zhu *et al.*, 2013b
Artemisia lancea	10 mg/ml	1,8-cineole (34.56%) & camphor (16.65%)	99%			
Arisaema franchetianum	10 mg/ml	Carvacrol & Linalool	99%			
Arisaema lobatum	10 mg/ml	Carvacrol & Linalool	99%			
Anacardium occidentale	1.72 mg/ml	β -ocimene (28.8%) α -copaene	95%			Ademola & Eloff, 2011
Chenopodium ambrosioide	1 mg/ml	ascaridole, carvacrol	100%			Salifou *et al.*, 2013
Myracrodruon urundeuva	0.29 mg/ml	α-Pinene (87.85%)			Reduced	Soares *et al.*, 2018

EHA: Egg hatch Assay; LDA: Larval Development Assay; LMA: Larval Motility Assay

Concluding Remarks

There has been an emerging interest by using plant essential oils to control *Haemonchus contortus* due to anthelminthic resistance issue in nematode population as well concerns about the residues in animal byproducts. Therefore using essential oils against *Haemonchus contortus* is profitable approach over anthelminthic drugs. The use of essential oils against *Haemonchosis* is limited due to their antinutritional, cytotoxic effects, under dose response and issues in commercial availability. However, their complexity in structure and approach in clarity of mechanism of action the essential oils are in discussion to improve control strategies for *Haemonchosis* in small ruminants.

ACKNOWLEDGEMENT

We are highly thankful to Higher Education Commission, Technology Development Fund Program for providing financial assistance under Project No. TDF02-106.

REFERENCES

Abbas A, Iqbal Z, Abbas RZ, Khan MK, Khan JA, Sindhu ZD, Mahmood MS and Saleemi MK, 2017a. *In vivo* anticoccidial effects of *Beta vulgaris* (sugar beet) in broiler chickens. **Microb Path** 111: 139 - 144. **https://doi.org/10.1016/j.micpath.2017.07.052**

Abbas A, Iqbal Z, Abbas RZ, Khan MK and Khan A, 2017b. Immunomodulatory activity of *Pinus radiata* extract against coccidiosis in broiler chicken. **Pak Vet J** 37: 145 - 149.

Adorjan B, Buchbauer G. 2010. Biological properties of essential oils: an updated review. **Flavour Fragr J** *25*: 407 - 426. **https://doi.org/10.1002/ffj.2024**

Araújo SA, Soares AMDS, Silva CR, Almeida Júnior EB, Rocha CQ, Ferreira ATDS, Perales J, Costa-Júnior LM. 2017. *In vitro* anthelmintic effects of *Spigelia anthelmia* protein fractions against *Haemonchus contortus*. **PLoS One** 15: e0189803. **https://doi.org/10.1371/journal.pone.0189803**

Ademola IO, Eloff JN. 2011. Anthelmintic efficacy of cashew (*Anarcadium occidentale* L.) on *in vitro* susceptibility of the ova and larvae of *Haemonchus contortus*. **Afr J Biotechnol** 10: 9700 - 9705. **https://doi.org/10.5897/ajb09.1706**

Akkari H, Ezzine O, Dhahri, S, B'chir F, Rekik M, Hajaji S, Gharbi M. 2015. Chemical composition, insecticidal and *in vitro* anthelmintic activities of *Ruta chalepensis* (*Rutaceae*) essential oil. **Ind Crops Prod** 74: 745 - 751. **https://doi.org/10.1016/j.indcrop.2015.06.008**

Ankri S, Mirelman D. 1999. Antimicrobial properties of allicin from garlic. **Microbes Infect** 1: 125 - 129. **https://doi.org/10.1016/s1286-4579(99)80003-3**

Athanasiadou S, Githiori J, Kyriazakis I. 2007. Medicinal plants for helminth parasite control: facts and fiction. **Animals** 1: 1392 - 1400. **https://doi.org/10.1017/s1751731107000730**

Bakkali, F, Averbeck S, Averbeck D, Idaomar M. 2008. Biological effects of essential oils: a review. **Food Chem Toxicol** 46: 446 - 475. **https://doi.org/10.1016/j.fct.2007.09.106**

Behnia, M, Haghighi A, Komeylizadeh H, Tabaei SJS, Abadi A. 2008. Inhibitory effects of Iranian *Thymus vulgaris* extracts on in vitro growth of *Entamoeba histolytica*. **Korean J Parasitol** 46: 153. **https://doi.org/10.3347/kjp.2008.46.3.153**

Besier B, Kahn L, DobsonR, Smith D. 2015. May Barbervax a new strategy for *Haemonchus* management. In Proceedings of the Australian Veterinary Association (AVA) Annual Conferences, Pan Pacific (NZVA and AVA) Veterinary Conference.

Besier RB, Love SCJ. 2003. Anthelmintic resistance in sheep nematodes in Australia: the need for new approaches. **Aust J Exp Agric** 43: 1383 - 1391. **https://doi.org/10.1071/ea02229**

Burke JM, Miller JE, Olcott DD, Olcott BM, Terrill TH. 2004. Effect of copper oxide wire particles dosage and feed supplement level on *Haemochus contortus*infection in lambs. **Vet Parasitol** 123: 235 - 243. **https://doi.org/10.1016/j.vetpar.2004.06.009**

Calo JR, Crandall PG, O'Bryan CA, Ricke SC. 2015. Essential oils as antimicrobials in food systems: A review. **Food Control** 54: 111 - 119. **https://doi.org/10.1016/j.foodcont.2014.12.040**

Castilho CV, Fantatto RR, Gaínza YA, Bizzo HR, Barbi NS, Leitão SG, Chagas ACS. 2017. *In vitro* activity of the essential oil from *Hesperozygis myrtoides* on *Rhipicephalus* (Boophilus) *microplus* and *Haemochus contortus*. **Rev Bras Farmacogn** 27: 70 - 76. **https://doi.org/10.1016/j.bjp.2016.08.005**

Chagas ACDS, Passos WM, Prates HT, Leite RC, Furlong J, Fortes ICP. 2002. Acaricide effect of *Eucalyptus* spp essential oils and concentrated emulsion on *Boophilus microplus*. **Braz J Vet Res Ann Sci** 39: 247 - 253. **https://doi.org/10.1590/s1413-95962002000500006**

Chagas ACS, Domingues LF, Fantatto RR, Giglioti R, Oliveira MC, Oliveira DH, Jacob RG. 2014. *In vitro* and *in vivo* acaricide action of juvenoid analogs produced from the chemical modification of *Cymbopogon* spp. and *Corymbia citriodora* essential oil on the cattle tick *Rhipicephalus* (Boophilus) *microplus*. **Vet parasitol** 205: 277 - 284. **https://doi.org/10.1016/j.vetpar.2014.06.030**

Christaki E, Florou-Paneri P, Giannenas I, Papazahariadou M, Botsoglou NA Spais AB. 2004. Effect of a mixture of herbal extracts on broiler chickens infected with *Eimeria tenella.* **Anim Res** 53: 137 - 144. **https://doi.org/10.1051/animres:2004006**

Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, WarmingtonJ. R, Wyllie SG. 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). **J Appl Microbiol** 88: 170 - 175. **https://doi.org/10.1046/j.1365-2672.2000.00943.x**

Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W, 2016. Essential oils chemical characterization and investigation of some biological activities: A critical review. **Medicines** 3: 2 - 16. **https://doi.org/10.3390/medicines3040025**

Drudge JH, Szanto J, Wyant ZN, Elam G. 1964. Field studies on parasite control in sheep: comparison of thia-bendazole, ruelene, and phenothiazine. **Am J Vet Res** 25: 1512 - 1518.

Echevarria FAM, Armour JL, Duncan JL. 1991. Efficacy of some anthelmintics on an ivermectin-resistant strain of *Haemonchus contortus* in sheep. **Vet Parasitol** 39: 279 - 284. **https://doi.org/10.1016/0304-4017(91)90044-v**

Ferreira LE, Benincasa BI, Fachin AL, Franca SC, Contini SS, Chagas AC, Beleboni RO. 2016. *Thymus vulgaris* L. essential oil and its main component thymol: Anthelmintic effects against *Haemochus contortus*from sheep. **Vet Parasitol** 228: 70 - 76. **https://doi.org/10.1016/j.vetpar.2016.08.011**

Giannenas I, Florou-Paneri P, Papazahariadou M, Christaki E, Botsoglou NA, Spais AB. 2003. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. **Arch Tierernahr** 57: 99 - 106. **https://doi.org/10.1080/0003942031000107299**

Gopal RM, Pomroy WE, West DM. 1999. Resistance of field isolates of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* to ivermectin. **Int J Parasitol** 29: 781 - 786. **https://doi.org/10.1016/s0020-7519(99)00032-6**

Grando TH, de Sá, MF, Baldissera MD, Oliveira CB, de Souza ME, Raffin, RP, Monteiro SG. 2016. *In vitro* activity of essential oils of free and nanostructured *Melaleuca alternifolia* and of terpinen-4-ol on eggs and larvae of *Haemochus contortus*. **J Helminth** 90: 377 - 382. **https://doi.org/10.1017/s0022149x15000401**

Hasegawa T, Takano F, Takata T, Niiyama M, Ohta T. 2008. Bioactive monoterpene glycosides conjugated with gallic acid from the leaves of *Eucalyptus globulus*. **Phytochemistry** 69: 747 - 753. **https://doi.org/10.1016/j.phytochem.2007.08.030**

Idris M, Abbas RZ, Masood S, Rehman T, Farooq U, Babar W, Hussain R, Raza A, Riaz U. 2017. The potential of antioxidant rich essential oils against avian coccidiosis. **World's Poult Sci** J 73: 89 - 104. **https://doi.org/10.1017/s0043933916000787**

Ijaz, M, Khan MS, Avais M, Ashraf K, Ali MM, Khan MZU. 2009. Infection rate and chemotherapy of various helminthes in diarrhoeic sheep in and around Lahore. **J Anim Plant Sci** 19: 13 - 16.

Ijaz M, Zaman MA, Mariam F, Farooqi SH, Aqib AI, Saleem S, Ghaffar A, Ali A, Akhtar R. 2018. Prevalence, hematology and chemotherapy of

gastrointestinal helminths in camels. **Pak Vet J** 38: 81 - 85. **https://doi.org/10.29261/pakvetj/2018.016**

Inaam EA, El Tayeb, IB, Sania AS, Hassan T. 2007. The effect of *Haemonchus contortus* infection and treatment with ivermectin on drug-metabolizing enzymes. **Res J Anim Vet Sci** 2: 66 - 71.

Katiki LM, Chagas ACS, Bizzo HR, Ferreira JFS, Amarante AFTD. 2011. Anthelmintic activity of *Cymbopogon martinii*, *Cymbopogon schoenanthus* and *Mentha piperita* essential oils evaluated in four different *in vitro* tests. **Vet Parasitol** 183: 103 - 108. **https://doi.org/10.1055/s-0031-1282954**

Katiki LM, Chagas ACS, Takahira RK, Juliani HR, Ferreira JFS, Amarante AFTD. 2012. Evaluation of *Cymbopogon schoenanthus* essential oil in lambs experimentally infected with *Haemochus contortus*. **Vet Parasitol** 186: 312 - 318. **https://doi.org/10.1016/j.vetpar.2011.12.003**

Katiki LM, Barbieri AME, Araujo RC, Veríssimo CJ, Louvandini JFS. 2017. Synergistic interaction of ten essential oils against *Haemonchus contortus in vitro*. **Vet Parasitol** 243: 47 - 51. **https://doi.org/10.1016/j.vetpar.2017.06.008**

Khan MN, Sajid MS, Rizwan HM, Qudoos A, Abbas RZ, Riaz M, Khan MK. 2017. Comparative efficacy of six anthelmintic treatments against natural infection of fasciola species in sheep. **Pak Vet J** 37: 65 - 68.

Khan AU, Tipu MY, Shafee M, Khan NU, Kiani MMT, Rafeeq M, Shah SIA. 2018. *In-ovo* antiviral effect of *Nigella sativa* extract against Newcastle Disease Virus in experimentally infected chicken embryonated eggs. **Pak Vet J** 38: 434 - 437.

Khater HF, Ali AM, Abouelella GA, Marawan MA, Govindarajan M, Murugan K, Abbas RZ, Vaz NP, Benelli G. 2018. Toxicity and growth inhibition potential of vetiver, cinnamon, and lavender essential oil and theiblends against larvae of the sheep blowfly, *Lucillia sericata*. **Int J Dermatol** 57: 449 - 457. **https://doi.org/10.1111/ijd.13828**

Kiran K, Saleem F, Awan S, Ahmad S, Ahmad S, Malik MAA, Akhtar B, Raza M, Peerzada S and Sharif A, 2018. Anti-inflammatory and anticancer activity of *Pteris cretica* whole plant extracts. **Pak Vet J** 38: 225 - 230. **https://doi.org/10.29261/pakvetj/2018.063**

LeJambre LF, Windon RG, Smith WD. 2008. Vaccination against Haemonchus contortus: performance of native parasite gut membrane glycoproteins in Merino lambs grazing contaminated pasture. **Vet Parasitol** 153: 302 - 312. **https://doi.org/10.1016/j.vetpar.2008.01.032**

Macedo ITF, Bevilaqua CML, Oliveira LMBD, Camurça-Vasconcelos ALF, Vieira LDS, Amóra SDS A. 2011. Evaluation of *Eucalyptus citriodora* essential oil on goat gastrointestinal nematodes. **Rev Bras Parasitol Vet** 20: 223 - 227. **https://doi.org/10.1590/s1984-29612011000300009**

Macedo IT, Bevilaqua CM, de Oliveira LM, Camurça-Vasconcelos AL, Vieira LDS, Oliveira FR, Nascimento NR. 2010. Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes. **Vet Parasitol** 173: 93 - 98. **https://doi.org/10.1016/j.vetpar.2010.06.004**

Mahmood MS, Amir HW, Abbas RZ, Rafique A, Aslam B. 2018. Evaluation of antiviral activity of *Azadirachta indica* (Neem) bark extract against Newcastle disease virus. **Pak Vet J** 38: 25 - 28. **https://doi.org/10.29261/pakvetj/2018.005**

Machado M, Dinis AM, Salgueiro L, Cavaleiro C, Custódio JB, do Céu Sousa M. 2010. Anti-Giardia activity of phenolic-rich essential oils: effects of *Thymbra capitata*, *Origanum virens*, *Thymus zygis* subsp. *sylvestris*, and *Lippia graveolens* on trophozoites growth, viability, adherence, and ultrastructure. **Parasitol Res** 106: 1205 - 1215. **https://doi.org/10.1007/s00436-010-1800-7**

Maciel MV, Morais SM, Bevilaqua CML, Silva RA, Barros RS, Sousa RN, Souza-Neto MA. 2010. Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*. **Vet Parasitol** 167: 1 - 7. **https://doi.org/10.1016/j.vetpar.2009.09.053**

Marie-Magdeleine C, Hoste H, Mahieu M, Varo H, Archimede H. 2009. *In vitro* effects of *Cucurbita moschata* seed extracts on

Haemonchus contortus. **Vet Parasitol** 161: 99 - 105. **https://doi.org/10.1016/j.vetpar.2008.12.008**

Mehmood F, Qasim M, Khan Z, Iqbal N, Mehmood S, Lateef M, Shahzadi P. 2011. *In vitro* evaluation of anthelmintic activity of essential oils from different parts of *Skimmia laureola* (DC.) Zucc. ex Walp., ver. Nair. **Pak J Bot** 43: 2915 - 2918.

Mehmood K, Zhang H, Sabir AJ, Abbas RZ, Ijaz M, Durrani AZ, Saleem MH, Rehman MU, Iqbal MK, Wang Y, Ahmad HI, Abbas T, Hussain R, Ghori MT, Ali S, Khan AU, Li J. 2017. A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. **Microb Path** 109: 253 - 262. **https://doi.org/10.1016/j.micpath.2017.06.006**

Mwamachi DM, Audho JO, Thorpe W, Baker RL. 1995. Evidence for multiple anthelmintic resistance in sheep and goats reared under the same management in coastal Kenya. **Vet Parasitol** 60: 303 - 313. **https://doi.org/10.1016/0304-4017(95)00794-1**

Olounladé PA, Azando EVB, Hounzangbé-Adoté MS, Ha TT, Leroy E, Moulis C, Valentin A. 2012. *In vitro* anthelmintic activity of the essential oils of *Zanthoxylum zanthoxyloides* and *Newbouldia laevis* against *Strongyloides ratti*. **Parasitol Res** 110: 1427 - 1433. **https://doi.org/10.1007/s00436-011-2645-4**

Pessoa LM, Morais SM, Bevilaquac CML, Luciano, JHS. 2002. Anthelmintic activity of essential oil of *Ocimum gratissimum Linn*. and eugenol against *Haemochus contortus*. **Vet Parasitol** 109: 59 - 63. **https://doi.org/10.1016/s0304-4017(02)00253-4**

Prichard R. 1994. Anthelmintic resistance. **Vet Parasitol** 54: 259 - 268.

Puspitasari S, Farajallah A, Erni Sulistiawati M. 2016. Effectiveness of ivermectin and albendazole against *Haemonchus contortus* in sheep in West Java, Indonesia. **Trop Life Sci Res** 27: 135.

Rahman WA. 1994. Resistance to benzimidazole anthelmintics by *Haemonchus contortus* in goats in peninsular Malaysia. **Vet Parasitol** 55: 155 - 157. **https://doi.org/10.1016/0304-4017(94)90067-1**

Ribeiro WLC, Macedo ITF, dos Santos JML, de Oliveira EF, Camurça-Vasconcelos ALF, de Paula HCB Bevilaqua, CML. 2013. Activity of chitosan-encapsulated *Eucalyptus staigeriana* essential oil on *Haemochus contortus*. **Exp Parasitol** 135: 24 - 29. **https://doi.org/10.1016/j.exppara.2013.05.014**

Rodríguez AV, Goldberg V, Viotti H, Ciappesoni G. 2015. Early detection of *Haemonchus contortus* infection in sheep using three different faecal occult blood tests. **Open Vet J** 5: 90 - 97.

Rolfe PF, Boray JC, Fitzgibbon C, Parsons G, Kemsley P, Sangster N. 1990. Closantel resistance in *Huemonchus contortus* from sheep. **Aust Vet J** 67: 29 - 31. **https://doi.org/10.1111/j.1751-0813.1990.tb07390.x**

Rustaiyan A, Masoudi S. 2011. Chemical constituents and biological activities of Iranian Artemisia species. **Phytochem Lett** 4: 440 - 447. **https://doi.org/10.1016/j.phytol.2011.07.003**

Sangster NC. 1999. Anthelmintic resistance: past, present and future. **Int J Parasitol** 29: 115 - 124.

Sangster NC, Gill J. 1999. Pharmacology of anthelmintic resistance. **Parasitol Today** 15: 141 - 146. **https://doi.org/10.1016/s0169-4758(99)01413-1**

Salifou S, Daga DF, Attindehou S, Deguenon R, Biaou CF. 2013. Antiparasitic effects of the water extract from *Chenopodium ambrosioides* L. (Chenopodiaceae) against some gastrointestinal nematodes in West African Long Legged goats. **J Parasitol Vec Biol** 5: 13 - 16.

Shaaban HA, El-Ghorab AH, Shibamoto T. 2012. Bioactivity of essential oils and their volatile aroma components. **J Essent Oil Res** 24: 203 - 212. **https://doi.org/10.1080/10412905.2012.659528**

Sivaraj S, Dorny P, Vercruysse J, Pandey VS. 1994. Multiple and multigeneric anthelmintic resistance on a sheep farm in Malaysia. **Vet Parasitol** 55: 159 - 165. **https://doi.org/10.1016/0304-4017(94)90068-x**

Soares AMS, Oliveira JTA, Rocha CQ, Ferreira ATS, Perales J, Zanatta AC, Vilegas W, Silva CR, Costa-Junior LM. 2018. *Myracrodruon urundeuva* seed exudates proteome and anthelmintic activity against *Haemonchus contortus*. **PLoS One** 13: e0200848. **https://doi.org/10.1371/journal.pone.0200848**

Upadhyay RK. 2010. Essential oils: anti-microbial, antihelminthic, antiviral, anticancer and antiinsect properties. **J Appl Biosci** 36: 1 - 22.

Uppal RP, Yadav CL, Godara, P, Rana ZS. 1992. Multiple anthelmintic resistance in a field strain of *Haemonchus contortus* in goats. **Vet Res Commun** 16: 195 - 198. **https://doi.org/10.1007/bf01839155**

Vercesi AE, Kowaltowski AJ, Grijalba MT, Meinicke AR, Castilho RF. 1997. The role of reactive oxygen species in mitochondrial permeability transition. **Bioscience Rep** 17: 43 - 52. **https://doi.org/10.1023/a:1027335217774**

Verzera V, Mondello L, Ragusa S, Dugo G. 2000. Essential oil from leaves of typical Mediterranean plants. Note II *Ruta chalepensis* L. **Essenze Deriv Agrum** 70: 207 - 210.

Walentowska J, Foksowicz-Flaczyk J. 2013. Thyme essential oil for antimicrobial protection of natural textiles. **Int Biodeterior Biodegradation** 84: 407 - 411. **https://doi.org/10.1016/j.ibiod.2012.06.028**

Waruiru RM, Ngotho JW, Mukiri JG. 1998. Multiple and multigeneric anthelmintic resistance on a sheep farm in Kenya. **Trop Anim Health Prod** 30: 159 - 166. **https://doi.org/10.1023/a:1005007602986**

Zahran HEDM, Abou-Taleb HK, Abdelgaleil SA. 2017. Adulticidal, larvicidal and biochemical properties of essential oils against *Culex pipiens* L. **J Asia Pac Entomol** 20: 133 - 139. **https://doi.org/10.1016/j.aspen.2016.12.006**

Zhao FW, Luo M, Wang YH, Li ML, Tang GH, Long CL. 2010. A piperidine alkaloid and limonoids from *Arisaema decipiens*, a traditional antitumor herb used by the Dong people. **Arch Pharmacal Res** 33: 1735 - 1739. **https://doi.org/10.1007/s12272-010-1104-6**

Zhu L, Dai JL, Yang L, Qiu J. 2013a. *In vitro* ovicidal and larvicidal activity of the essential oil of *Artemisia lancea* against *Haemochus contortus* (Strongylida). **Vet Parasitol** 195: 112 - 117. **https://doi.org/10.1016/j.vetpar.2012.12.050**

Zhu L, Dai J, Yang L, Qiu J. 2013b. Anthelmintic activity of *Arisaema franchetianum* and *Arisaema lobatum* essential oils against *Haemochus contortus*. **J Ethnopharmacol** 148: 311 - 316. **https://doi.org/10.1016/j.jep.2013.04.034**