ISSN 2675-6617

In vitro efficacy of essential oils against *Haematobia irritans*

[Eficácia *in vitro* de óleos essenciais contra *Haematobia irritans*]

"Scientific Article/Artigo Científico"

Karina Neoob de Carvalho **Castro**[1], Lívio Martins **Costa-Júnior**[2], Kirley Marques **Canuto**[3], Ivanilza Moreira de **Andrade**[4], Alessandro Pelegrine **Minho**[5], Francisco de Assis Leite **Souza**[6]*

[1]Embrapa Gado de Leite, Juiz de Fora-MG, Brasil.
[2]Centro de Ciências Biológicas e da Saúde, Universidade Federal do Maranhão, São Luís-MA, Brasil.
[3]Embrapa Agroindústria Tropical, Fortaleza-CE, Brasil.
[4]Universidade Federal do Delta do Parnaíba, Parnaíba-PI, Brasil.
[5]Embrapa Pecuária Sudeste, São Carlos-SP, Brasil.
[6]Laboratório de Diagnóstico Animal, Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Recife-PE, Brasil.
*Corresponding author/Autor para correspondência: E-mail: francisco.alsouza@ufrpe.br

Abstract
The horn fly (*Haematobia irritans*) infestation in the cattle in Brazil leads to significant economic damage for livestock. The efficacy of many essential oils has been demonstrated in the control of veterinary ectoparasites. This study aimed to evaluate the *in vitro* efficacy of four essential oils to adult horn fly control. The essential oil from *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae) was extracted by hydro-distillation, while that the water steam distillation method was used to extract the essential oils from *Varronia curassavica* (Jacq.) Roem. & Schult. (Boraginaceae), *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm. (Zingiberaceae) and *Psidium guajava* L. (Myrtaceae). Afterwards, all the essential oils were analyzed by Gas Chromatography Mass Spectrometry (GC-MS) and Flame Ionization Detector (GC-FID) for determination of their chemical composition. The bioassays *in vitro* were performed according to the impregnated filter paper methodology. All the essential oils evaluated in this study exhibited efficacy *in vitro* against *H. irritans.* The highest mortality of *H. irritans* was determined by essential oils from *A. zerumbet* (100%) and *M. suaveolens* (96.2%) at 50 mg mL^{-1}. In concentration of 100 mg mL^{-1} *V. curassavica* and *P. guajava* essential oils caused the mortality of 99.2% and 82.8%, respectively. The *A. zerumbet*, *M. suaveolens, V. curassavica* and *P. guajava* essential oils showed *in vitro* efficacy against *H. irritans.* Further studies with associations and nanoemulsions are necessary to increase the efficacy and stability of these essential oils on field conditions.

Keywords: control; insecticidal; plants.

Resumo
A infestação pela mosca-dos-chifres (*Haematobia irritans*) em bovinos no Brasil leva a prejuízos econômicos significativos para a pecuária. A eficácia de muitos óleos essenciais foi demonstrada no controle de ectoparasitas de interesse veterinário. O objetivo deste estudo foi avaliar a eficácia *in vitro* de quatro óleos essenciais no controle da mosca-dos-chifres. O óleo essencial de *Mesosphaerum suaveolens* (L.) Kuntze (Lamiaceae) foi extraído por hidrodestilação, enquanto o método de destilação a vapor de água foi usado para extrair os óleos essenciais de *Varronia curassavica* (Jacq.) Roem & Schult. (Boraginaceae), *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm. (Zingiberaceae) e *Psidium guajava* L. (Myrtaceae). Em seguida, todos os óleos essenciais foram analisados por Espectrometria de Massa por Cromatografia Gasosa (CG-EM) e Detector de Ionização de Chama (CG-DIC) para determinação da sua composição química. Os bioensaios *in vitro* foram realizados de acordo com a metodologia do papel de filtro impregnado. Todos os óleos essenciais avaliados neste estudo exibiram eficácia *in vitro* contra *H. irritans.* A mais elevada mortalidade de *H. irritans* foi determinada pelos óleos essenciais de *A. zerumbet* (100%) e *M. suaveolens* (96.2%) a 50 mg mL^{-1}. Na concentração de 100 mg mL^{-1}os óleos essenciais de *V. curassavica*e *P. guajava* causaram a

Received 16 November 2022. Accepted 09 January 2023.
DOI: https://doi.org/10.26605/medvet-v16n4-5357

mortalidade de 99,2% e 82,8%, respectivamente. Os óleos essenciais de *A. zerumbet, M. suaveolens, V. curassavica* e *P. guajava* mostraram eficácia *in vitro* contra *H. irritans.* Mais estudos com associações e nanoemulsões são necessários para aumentar a eficácia e estabilidade destes óleos essenciais frente às condições de campo.

Palavras-chave: controle; inseticidas; planta.

Introduction

The horn fly (*Haematobia irritans*) infestation in Brazilian cattle herd leads to significant economic damage for livestock, mainly due to the loss on average 10% of the life weight of the animals. The importance of this parasite in livestock production is due to the high level of stress related to multiple bites, decreased appetite, anemia and change in the quality of the leather of cattle, in addition of transmission of disease to the animals (Bianchin et al., 2004; Muñoz and Serrano, 2007). The development and maintenance of ectoparasites occur throughout the year in most areas of the country because climatic conditions are generally favorable (Costa et al., 2014).

Chemical control of horn fly has been pointed out as an essential part of the livestock management, but the farmer lacks guidance in choosing the insecticide used as treatment (Barros et al., 2007; Costa et al., 2016). Besides damaging the environmental the indiscriminate use of insecticides, increases production costs and leas to the accumulation of residues in meat and milk (Rübensam et al., 2013). This fact has contributed to the selection of population resistant of this parasite to the products available on the market (Maciel et al., 2015). This gradual increase of the difficulty of control of this parasite will tend to increase the population levels of horn fly (Bianchin et al., 2004).

Pesticides used in the control of parasites in farm animals are potentially toxic to humans. The grace period for these products is sometimes not to respected, leaving residues in the meat and milk of these animals (Rothwell et al., 2001; Nero et al., 2007). Dairy cattle can accumulate such residues in milk from direct (ectoparasite treatment) or indirect (animal feed and water) contamination (Pagliuca et al., 2006). Milk samples contaminated with pesticide residues have been detected in several regions of Brazil, which is a risk to consumer health (Nero et al., 2007). Although not approved for use in lactating cows, some chemicals products have been used indiscriminately in Brazil, since the control of these residues in the country is still incipient (Castro et al., 2012). In this context, it is important seek alternatives of natural origin to control bovine ectoparasites, as a way to ensure the sustainable production of health foods.

It has been well documented that a wide variety of active ingredients of plant origin are naturally available for use in parasite control (Chagas, 2004). The efficacy of many essential oils in the control of veterinary ectoparasites has been demonstrated (Ellse and Wall, 2014). The essential oil of *Mesosphaerum suaveolens* (L.) Kuntze and *Varronia curassavica* (Jacq.) Roem. & Schult., native species and *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm. and *Psidium guajava* L., adapted and widely cultivated in Brazil, showed efficacy against larvae and engorged females of *Rhipicephalus microplus* (Castro et al., 2018; Castro et al., 2019). Considering the potential of these species, this study aimed to evaluate the *in vitro* efficacy of *M. suaveolens, V. curassavica, A. zerumbet* and *P. guajava* essential oils to the horn fly control.

Material and Methods

Plant material

M. suaveolens (Lamiaceae), *V. curassavica* (Boraginaceae), *A. zerumbet* (Zingiberaceae) and *P. guajava* (Myrtaceae) are species known, respectively, as “bamburral”, “erva-baleeira”, “colônia” and “goiabeira”. *M. suaveolens* was harvested in July 2013 at the Embrapa Meio-Norte (3°05’24.0”S, 41°46’12.5”W), while that *V. curassavica* (03°01’41”S, 41°44’56”W) and *A. zerumbet* (03°01’27.5”S, 41°44’53.5”W) were collected at Fazenda Tabuleiros II of the Anidro do Brasil Extrações S.A., respectively in June 2012 and July 2011. *P. guajava* was harvested from farms at Tabuleiros Litorâneos (03°01’18”S, 41°46’92”W) in June 2012. All the species were collected in the municipality of Parnaíba, Piauí state, Brazil. Voucher specimens were deposited in the Herbarium (CEN) of the *Embrapa Recursos Genéticos e Biotecnologia*, under registration numbers 81.097 (*M. suaveolens*), 81.102 (*V.*

curassavica), 81.103 (*A. zerumbet*) and 81.100 (*P. guajava*).

The essential oils were extracted at the Embrapa Meio-Norte, in Parnaíba, Piauí, from the aerial parts of *M. suaveolens* by hydro-distillation using a Clevenger apparatus. Nearly 2 kg of fresh plant was mixed with 3 L of water and boiled for 3 h. A water steam distillation method was used to extract the essential oil from the leaves of *A. zerumbet*, *V. curassavica* and *P. guajava*. The essential oils obtained were stored at 4 °C in a freezer. An aliquot of each sample was tested and analyzed two months after the collect, in at the Embrapa Agroindústria Tropical, in Fortaleza, Ceará, by Gas Chromatography Mass Spectrometry (GC-MS) and Flame Ionization Detector (GC-FID) for determination of their chemical composition (Castro et al., 2016).

Essential oil analysis

The GC–MS analyses were carried out on an Agilent 7890B-GC/5977A-MS instrument equipped with a nonpolar VF-5MS fused silica capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness) utilizing a split ratio of 1:30 and helium at 1.5 mL min-1 as the carrier gas. The injector temperature and detector temperature were set at 250 °C. The oven temperature was raised from 70 to 180 °C at 4 °C min-1 and afterwards to 250 °C at 10 °C min-1. Mass spectra were recorded in a range of mass-to-charge ratio (m/z) between 30 and 450. GC-FID analyses were performed on a Shimadzu GC-2010 Plus chromatograph in the same aforementioned chromatographic conditions except for the carrier gas (hydrogen). The retention indices were determined by the injection of a mixture of C7-C30 homologous n-alkanes (Sigma, St. Louis, MO). The identification of the volatile compounds was achieved through the comparison of the mass spectra recorded with those provided by the spectrometer database (NIST 02–287,324 compounds) along with the retention indices and mass spectra of the literature (Adams, 2007; NIST, 2011). The relative content of each constituent was quantified as the normalized peak area detected in the GC-FID chromatogram and expressed as a percentage.

The chemical composition of the essential oils from *A. zerumbet* (Castro et al., 2016), *M. suaveolens* (Castro et al., 2018) and *V. curassavica* and *P. guajava* (Castro et al., 2019) were previously determined. Twenty-one and twenty-three components were identified in the essential oils from *A. zerumbet* and *M. suaveolens*, respectively, while those thirty-three and thirty components were identified, respectively, in the *V. curassavica* and *P. guajava* essential oils. The most abundant components found in the essential oils were: 1,8-cineole (35.8%) and sabinene (19.6%) in *M. suaveolens;* p-cymene (32.7%), 1,8-cineole (24.0%) and terpinen-4-ol (20.2%) in *A. zerumbet;* α-pinene (49.0%) and the β-caryophyllene (12.4%) in *V. curassavica* and β-caryophyllene (38.9%), β-selinene (9.7%) and α-selinene (9.7%) in *P. guajava*.

Bioassay of impregnated filter paper

For each essential oil, solutions were prepared at concentrations of 6.2, 12.5, 25.0, 50.0 and 100.0 mg/mL. The negative controls consisted of the solvents used in the essential oil dilutions, which were 50% ethanol and 3% Tween 80. All tests were replicated three times.

The bioassays *in vitro* were carried out at the Unidade de Execução de Pesquisa de Parnaíba da Embrapa Meio-Norte, according to the impregnated filter paper methodology proposed by Sheppard and Hinkle (1987) and adapted by Barros et al. (2002), in November, in the year respective in which each plant was collected. Filter paper discs with 9 cm of diameter were impregnated with 1 mL of solutions of the essential oils diluted in 50% ethanol and 3% tween 80. After drying the paper filter at room temperature, these were placed in disposable Petri dishes for mounting of the kits. Each dishes contained a single hole (about Ø1 cm) made at the center of the bottom (facing up) to permit fly loading.

Adult flies were captured in the morning with hand nets on naturally infested Nellore x Holstein cattle (no use of insecticides for three months) and transferred to the kits. About 90 flies were distributed in three petri dishes per concentration, which were sent to the laboratory of Parasitology of the Embrapa Meio-Norte. Mortality was assessed immediately after dishes were loaded, to check for any early mortality caused by fly manipulation, and after 2 h of exposure to the treatment. Flies unable to walk were considered dead.

Mortality data were corrected by Abbott’s formula (Abbott, 1925) when necessary and analyzed by POLO-PC (LeOra, 1987) with fly mortality at the control dishes higher than 10% were excluded.

Results and Discussion

All the essential oils evaluated in this study exhibited efficacy *in vitro* against the adult horn fly (Table 1). The highest mortality of *H. irritans* was determined by essential oil from *A. zerumbet* (100%), followed by *M. suaveolens* essential oil (96.2%) at 50 mg mL^{-1}. In the concentration of 100 mg mL^{-1}, these essential oils caused 100% of mortality to *H. irritans*, while that those from *V. curassavica* and *P. guajava* caused the mortality of 99.2% and 82.8%, respectively.

Table 1. *In vitro* activity of *Alpinia zerumbet*, *Mesosphaerum suaveolens*, *Varronia curassavica*, *Psidium guajava* essential oils on adult fly of *Haematobia irritans*.

Treatment	Concentration (mg mL^{-1})	Mortality (%)
Negative control	-	$0.6 \pm 1.1^{b,C}$
Alpinia zerumbet	6.2	$0.0 \pm 0.0^{b,C}$
	12.5	$1.7 \pm 2.9^{b,C}$
	25.0	$92.4 \pm 7.7^{a,A,B}$
	50.0	$100.0 \pm 0.0^{a,A}$
	100.0	$100.0 \pm 0.0^{a,A}$
Mesosphaerum suaveolens	6.2	$0.0 \pm 0.0^{b,C}$
	12.5	$1.3 \pm 2.2^{b,C}$
	25.0	$15.9 \pm 5.7^{b,C}$
	50.0	$96.2 \pm 3.8^{a,A}$
	100.0	$100.0 \pm 0.0^{a,A}$
Varronia curassavica	6.2	$0.0 \pm 0.0^{b,C}$
	12.5	$0.0 \pm 0.0^{b,C}$
	25.0	$1.6 \pm 1.4^{b,C}$
	50.0	$7.3 \pm 3.5^{b,C}$
	100.0	$99.2 \pm 1.4^{a,A}$
Psidium guajava	6.2	$0.8 \pm 1.4^{b,C}$
	12.5	$0.0 \pm 0.0^{b,C}$
	25.0	$6.2 \pm 5.8^{b,C}$
	50.0	$11.3 \pm 7.4^{b,C}$
	100.0	$82.8 \pm 19.3^{a,B}$

Values represent average ± standard deviation. Negative control (ultrapure water and 50% ethanol + 1% tween 80). Different lowercase letters represent a difference statistically significant ($p \leq 0.05$) among concentrations of the same oil. Different capital letters represent a difference statistically significant ($p \leq 0.05$) among all concentrations tested.

Four plant species were selected for this study because their essential oils were effective against the ectoparasite *Rhipicephalus microplus* (Castro et al., 2016; Castro et al., 2018; Castro et al., 2019). This is the first study describing the *in vitro* efficacy of *A. zerumbet, M. suaveolens, V. curassavica* and *Psidium guajava* essential oils on adult horn fly (*H. irritans),* a sanitary problem of great importance for Brazilian livestock (Bianchin et al., 2004; Muñoz and Serrano, 2007). Although the results have been promising, there was a significant ($p \leq 0.05$) drop in mortality of *H. irritans*, when they were treated with the essential oils in the concentrations of 12.5 mg mL^{-1} (*A. zerumbet*), 25.0 mg mL^{-1} (*M. suaveolens*) and 50 mg mL^{-1} (*V. curassavica* and *P. guajava*).

A. zerumbet, whose essential oil was the most efficient against *H. irritans*, belongs to the family Zingiberaceae, as well as *Curcuma longa* (L.). The essential oil from this latter species caused mortality of 96.66% in the third instar larvae of *Cochliomyia macellaria* (Chaaban et al., 2019), one of the species of flies responsible for cutaneous myiasis. Both species posses p-cymene and 1,8-cineole as major compounds in their essential oils. 1,8-cineole is the main constituent present in various essential oils from genus Eucalyptus, that are toxic to *H. irritans* and also contains p-cymene (Juan et al., 2011). The predominance of 1,8-cineole in *M. suaveolens* essential oil also was verified. It is possible that the presence of a high amount of 1,8-cineole contributes for the best results observed in this study. Also, Castro et al. (2018) observed that essential oils from *M. suaveolens*, *A. zerumbet* and *Ocimum gratissimum* L. contained intermediary concentrations of 1,8-cineole and were effective on engorged females and larvae of tick *R. microplus*.

In this study, *V. curassavica* and *P. guajava* essentials oils caused respectively, the mortality of 99,2% and 82,8% in *H. irritans*, only when the concentration was maximum (100 mg mL^{-1}), drastically decreasing its action, when the concentrations were reduced to half. Currently, associations and other strategies are used to increase efficiency of these products. Associations between *V. curassavica* (synonym = *Cordia verbenacea* DC. and *Cordia curassavica* (Jacq.) Roem. & Schult.) and *P. guajava* essential oils plus cashew nutshell liquid displayed significant effect in both stages of life of *R. microplus* (Castro et al., 2019). Otherwise, the extract of *Cordia myxa* L. associated with cadmium oxide nanoparticles demonstrated that synergistics effects on pupa of *Musca domestica* can be obtained with the use of nanoemulsions (Foad et al., 2020).

The major constituent of *V. curassavica* is α-pinene. This compound, as well as β-caryophyllene, other principal constituent from *V. curassavica* and *P. guajava*, exhibited great contact toxicity to three stored insects (Sun et al., 2020). β-cariophyllene is also the major constituent from *Lantana montevidensis* (Spreng.) Briq. essential oil, which proved to be toxic against the fly *Drosophila melanogaster*, causing significant damage to your locomotor system (Bezerra et al., 2017). The action mechanism of essential oils on insects can be diverse, however the fumigant activity has been the most commonly reported. According to Juan et al. (2011), the volatility of the essential oils favors the penetration of toxic substances through the insect spiracles as part of the respiratory process. The pulverization with essential oil on cows infested with *H. irritans* can repel the flies in the first hours after application (Boito et al., 2018). However, the typical of the essential oil can lead to degradation and loss of the stability in the natural environment (Turek and Stintzing, 2013).

Another research line is to use the essential oils to potentiate the insecticidal effect of chemical drugs. These associations could maximize the total antiparasitic effect of the commercial insecticide. According to Fazolin et al. (2016), the *use* of synergists (between essential oil and commercial insecticides) is important in minimizing the amount of *chemical insecticide* required for insect control, leading to lower environmental impacts.

Conclusion

The *A. zerumbet*, *M. suaveolens*, *V. curassavica* and *P. guajava* essential oils evaluated *in vitro* are effective against *H. irritans*, however, in the farms, the climatical conditions can impair their effectiveness. Therefore, are necessary further studies with associations between essential oils and nanoemulsions to increase the bioactivity and stability of these formulations in parasitized animals on field conditions.

Conflict of Interest

The authors declare no conflict of interest

Ethics Committee

The execution of this research dispensed with the need to obtain ethical licenses, since we worked with invertebrate animals (horn fly).

Acknowledgements

The authors are grateful to João Batista Alves de Souza for collaboration in experiments.

References

Abbott, W.S. A method of computing the effectiveness of an insecticide. **Journal of Economic Entomology**, 18(2): 256-257, 1925.

Adams, R.P. **Identification of essential oil components by gas chromatography/ mass spectroscopy**. 4th ed. Illinois: Allured, 2007. 804p.

Barros, A.T.M. Desenvolvimento de *Haematobia irritans* em massas fecais de bovinos mantidas em laboratório. **Pesquisa Agropecuária Brasileira**, 37(2): 217-221, 2002.

Barros, A.T.M.; Gomes, A.; Koller.W.W. Insecticide susceptibility of horn lies, *Haematobia irritans* (Diptera: Muscidae), in the state of Mato Grosso do Sul, Brazil. **Revista Brasileira de Parasitologia Veterinária**, 16(3): 145-151, 2007.

Bezerra, J.W.A.; Rodrigues, F.C.; Costa, A.R.; Boligon, A.A.; Rocha, J.B.T.; Barros, L.M. Estudo químico-biológico do óleo essencial de *Lantana montevidensis* (chumbinho) (Spreng.) Briq. (Verbenaceae) contra *Drosophila melanogaster*. **Revista Cubana de Plantas Medicinales**, 22(1): 1-13, 2017.

Bianchin, I.; Koller, W.W.; Alves, R.G.O.; Detmann, E. Efeito da mosca-dos-chifres, *Haematobia irritans* (L.) (Diptera: Muscidae), no ganho de peso de bovinos Nelore. **Ciência Rural**, 34(3): 885-890, 2004.

Boito, J.P.; Silva, A.S.; Reis, J.H.; Santos, D.S.; Gebert, R.R.; Biazus, A.H.; Santo, R.C.V.; Quatrin, P.M.; Ourique, A.F.; Boligon, A.A.; Baretta, D.; Baldissera, M.D.; Stefani, L.M.; Machado, G. Insecticidal and repellent effect of cinnamon oil on flies associated with livestock. **Revista MVZ Córdoba**, 23(2): 6628-6636, 2018.

Castro, K.N.C.; Teodoro, M.S.; Costa, J.V.; Nascimento, E.S.; Szabo, M.P.J.; Magalhães, J.A.; Barbosa, A.M.R. Bovinocultura leiteira de agricultores familiares de Parnaíba-PI. **Pubvet**, 6(6): 1295, 2012.

Castro, K.N.C.; Lima, D.F.; Vasconcelos, L.C.; Santos, R.C.; Pereira, A.M.L.; Fogaça, F.H.S.; Canuto, K.M.; Brito, E.S.; Calvet, R.M. Composição química e eficácia do óleo essencial e do extrato etanólico de *Alpinia zerumbet* sobre *Staphylococcus aureus*.

Arquivos do Instituto Biológico, 83(1): 1-7, 2016.

Castro, K.N.C.; Canuto, K.M.; Brito, E.S.; Costa-Júnior, L.M.; Andrade, I.M.; Magalhães, J.A.; Barros, D.M.A. Eficácia *in vitro* de óleos essenciais com diferentes concentrações de 1,8-cineol contra *Rhipicephalus (Boophilus) microplus*. **Revista Brasileira de Parasitologia Veterinária**, 27(2): 203-210, 2018.

Castro, K.N.C.; Costa-Júnior, L.M.; Lima, D.F.; Canuto, K.M.; Brito, E.S.; Andrade, I.M.; Teodoro, M.S.; Oiram-Filho, F.; Santos, R.C.; Mayo, S.J. Acaricidal activity of cashew nut shell liquid associated with essential oils from *Cordia verbenacea* and *Psidium guajava* on *Rhipicephalus microplus.* **Journal of Essential Oil Research**, 31(4): 297-304, 2019.

Chaaban, A.; Gomes, E.M.; Richardi, V.S.; Martins, C.E.N.; Brum, J.S.; Navarro-Silva, M.A.; Deschamps, C.; Molento, M.B. Essential oil from *Curcuma longa* leaves: Can an overlooked by-product from turmeric industry be effective form myiasis control? **Industrial Crops and Products**, 132(2): 352-364, 2019.

Chagas, A.C.S. Controle de parasitas utilizando extratos vegetais. **Revista Brasileira de Parasitologia Veterinária**, 13(1): 156-160, 2004.

Costa, M.S.F.; Guimarães, M.P.; Lima, W.S.; Costa, A.J.F.; Facury Filho, E.J.; Araújo, R.N. Seasonal Variation and Frequency Distribution of Ectoparasites in Crossbreed Cattle in Southeastern Brazil. **Journal of Veterinary Medicine**, 2014 (ID 759854): 1-8, 2014.

Costa, E.G.L.; Carneiro, J.C.; Bastos, G.A.; Vasconcelos, V.O.; Souza, R.M.; Almeida, A.C.; Duarte, E.R. Controle de *Haematobia irritans* no Semiárido de Minas Gerais. **Acta Scientiae Veterinariae**, 44(1): 1-10, 2016.

Ellse, L.; Wall, R. The use of essential oils in veterinary ectoparasite control: a review. **Medical and Veterinary Entomology**, 28(3): 233-243, 2014.

Fazolin, M.; Estrela, J.L.V.; Monteiro, A.F.M.; Silva, I.M.; Gomes, L.P.; Silva, M.S.F. Combining the essential oil of *Piper aduncum* L. with commercial insecticides. **Semina: Ciências Agrárias**, 37(6): 3903-3914, 2016.

Foad, Z.A.; Al-Bahadyli, L.J.M.; Ahmed, N.A. The synergistic effect of cdo nps concentration and crude fruit extract of *Cordia myxa* concentration on pupa of *Musca domestica*. **Plant Archives**, 20(1): 2849-2854, 2020.

Juan, L.W.; Lucia, A.; Zerba, E.N.; Harrand, L.; Marco, M.; Masuh, H.M. Composição Química e Toxicidade Fumigante dos Óleos Essenciais de 16 Espécies de Eucalipto Contra *Haematobia irritans* (Diptera: Muscidae) Adultos. **Journal of Economic Entomology**, 104(3): 1087-1092, 2011.

LeOra Software. **POLO-PC: user's guide to Probit Logit analysis.** Bekerly: Leora Software, 1987.

Maciel, W.G.; Lopes, W.D.Z.; Cruz, B.C.; Teixeira, W.F.P.; Felippelli, G.; Sakamoto, C.A.M.; Fávero, F.C.; Buzzulini, C.; Soares, V.E.; Gomes, L.V.C.; Bichuette, M.A.; Costa, A.J. Effects of *Haematobia irritans* infestation on weight gain of Nelore calves assessed with different antiparasitic treatments chemes. **Preventive Veterinary Medicine**, 118(1): 182-186, 2015.

Muñoz, M.M.; Serrano, R.E. Infestación por *Haematobia irritans* enel toro de lidia (mosca de loscuernos). **Revista Complutense de Ciencias Veterinarias**, 1(2): 347-351, 2007.

NIST. National Institute of Standards and Technology. **Mass spectral library (NIST/EPA/NIH).** Gaithersburg; 2011. Available at: <http://webbook.nist.gov/chemistry/>. Accessed on: 22 mar. 2022.

Nero, L.A.; Mattos, M.R.; Beloti, V.; Barros, M.A.F.; Netto, D.P.; Franco, B.D.G.M. Organofosforados e carbamatos no leite produzido em quatro regiões leiteiras no Brasil: ocorrência e ação sobre *Listeria monocytogenes* e *Salmonella* spp. **Ciência e Tecnologia de Alimentos**, 27(1): 201-204, 2007.

Pagliuca, G.; Serraino, A.; Gazzotti, T.; Zironi, E.; Borsari, A.; Rosmini, R. Organophosphorus pesticides residues in Italian raw milk. **Journal of Dairy Research**, 73(3): 340-344, 2006.

Rothwell, J.T.; Burnett, T.J.; Hacket, K.; Chevis, R.; Lowe, L.B. Residues of zeta-cypermethrin in bovine tissues and milk following pour-on and spray application. **Pest Management Science**, 57(11): 993-999, 2001.

Rübensam, G.; Barreto, F.; Hoff, R.B.; Pizzolato, T.M. Determination of avermectin and milbemycin residues in bovine muscle by liquid chromatography-tandem mass spectrometry and fluorescence detection using solvent extraction and low temperature cleanup. **Food Control**, 29(1): 55-60, 2013.

Sheppard, D.C.; Hinkle, N.C. A field procedure using disposable materials to evaluate horn fly insecticide resistance. **Journal of Agricultural Entomology**, 4(1): 87-89, 1987.

Sun, J.S.; Feng, Y.; Wang, Y.; Li, J.; Zou, K.; Liu, H.; Hu, Y.; Xue, Y.; Yang, L.; Du, S.; Wu, Y. α-Pinene, Caryophyllene and β-Myrcene from *Peucedanum terebinthaceum* Essential oil: Insecticidal and Repellent Effects on Three Stored-Product Insects. **Records of Natural Products**, 14(3): 177-189, 2020.

Turek, C.; Stintzing, F.C. Stability of Essential Oils: A Review. **Comprehensive Reviews in Food Science and Food Safety**, 12(1): 40-53, 2013.