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Effects of essential oil of *Eucalyptus globulus* loaded in nanoemulsions and in nanocapsules on reproduction of cattle tick *(Rhipicephalus microplus)*

Galli, G.M.[1]; Volpato, V.[1]; Santos, R.C.V.[2]; Gebert, R.R.[1]; Quatrin, P.[3]; Ourique, A.F.[3]; Klein, B.[4]; Wagner, R.[4]; Tonin, A.A.[5]; Baldissera, M.D.[2]; Stefani, L.M.[1] and Da Silva, A.S.[1@]

[1]Graduate Program of Animal Science and Department of Animal Science, Universidade do Estado de Santa Catarina, Chapecó, SC, Brazil.
[2]Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.
[3]Laboratory of Nanotechnology, Programa de Pós-Graduação em Nanociências, UNIFRA, Santa Maria, Rio Grande do Sul, Brazil.
[4]Department of Food Technology, Universidade Federal de Santa Maria, RS, Brazil.
[5]Mestrado em Sanidade e Produção Animal Aplicados a Pequenas Propriedades, Universidade do Oeste de Santa Catarina, UNOESC, Xanxerê, Santa Catarina, Brazil.

SUMMARY

Infestation of cattle tick *(Rhipicephalus microplus)* produces important lost in the livestock farming. The aim of this study was to investigate whether essential oil of *Eucalyptus globulus* in different nanosystems (nanoemulsions and nanocapsules) is able to potentiate the negative effect on cattle tick reproduction, interfering on its oviposition and eggs hatchability. Engorged females of *R. microplus* were obtained from cows naturally infested. In experimental conditions, these females were immersed in tests solutions at concentrations of 1, 5 and 10%, containing pure eucalypt essential oil; and solutions of it in nanocapsules and nanoemulsions, at concentrations of 0.5, 1 and 5%. Tests using pure eucalypt essential oil, at 5 and 10% concentrations, showed 85 and 97.8% of efficacy, respectively. However, the oil encapsulated was not able to potentiate the carrapaticide effect, presenting 61.2% and 50% of efficacy (at 5%), using nanoemulsions and nanocapsules. Therefore, our results allowed us to conclude that essential oil of *E. globulus*, in its pure form, interfered negatively on reproduction of *R. microplus*; however, the nanostructured forms had low efficacy.

ADDITIONAL KEYWORDS

Livestock.
Tick.
Natural products.
Essential oil.
Nanotechnology.

Efectos del aceite esencial de *Eucalyptus globulus* cargado en nanoemulsiones y en nanocápsulas en la reproducción de la garrapata bovina *(Rhipicephalus microplus)*

RESUMEN

La infestación por garrapatas *(Rhipicephalus microplus)*, genera importantes pérdidas en la producción bovina. El objetivo de este estudio fue investigar si el aceite esencial de *Eucalyptus globulus* en diferentes nanosistemas (nanoemulsiones y nanocápsulas) es capaz de potenciar el efecto negativo sobre la reproducción de la garrapata, interfiriendo en su oviposición y eclosión de los huevos. Las hembras engordadas de *R. microplus* se obtuvieron de vacas naturalmente infestadas. En condiciones experimentales, estas hembras fueron sumergidas en soluciones de ensayo a concentraciones de 1, 5 y 10%, conteniendo aceite esencial puro de eucalipto; Y soluciones de la misma en nanocápsulas y nanoemulsiones, a concentraciones de 0,5, 1 y 5%. Las pruebas con aceite esencial de eucalipto puro, a concentraciones de 5 y 10%, mostraron 85 y 97,8% de eficacia, respectivamente. Sin embargo, el aceite encapsulado no fue capaz de potenciar el efecto carrapaticida, presentando 61,2% y 50% de eficacia (al 5%), utilizando nanoemulsiones y nanocápsulas. Por lo tanto, nuestros resultados nos permitieron concluir que el aceite esencial de *E. globulus*, en su forma pura, interfería negativamente en la reproducción de *R. microplus*; Sin embargo, las formas nanoestructuradas tenían baja eficacia.

PALABRAS CLAVE ADICIONALES

Ganado.
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INFORMATION

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Correspondencia a los autores/Contact e-mail:
aleksandro_ss@yahoo.com.br

INTRODUCTION

Nanotechnology is a multidisciplinary area, possessing applicability in different industrial sectors, including on animal health and livestock production. In this sense, many nanostructured systems have been employed, such as liposomes, nanoemulsions, lipid nanoparticles and polymeric nanoparticles, most of them in attempt to improve the therapeutic efficacy of many antiparasitic drugs (Mishra et al., 2010). According to these authors, the nanotechnology has many advantages, such as the improvement of efficacy and biodisponibility, reduction of colateral effects and increase of drug stability. A recently study conducted by Pazinatto et al. (2014) demonstrated that nanocapsules containing essential oil of *Melaleuca alternifolia* was able to potentiates the acaricide effects, presenting 100% of efficacy on cattle tick, even in low doses.

The cattle tick *Rhipicephalus (Boophilus) microplus* is a hematophagous ectoparasite that causes severe economic losses on livestock, since it is considered an important vector of diseases (Constantinoiu et al., 2010). Synthetic carrapaticides are used in controlling of *R. microplus;* however, several cases of resistance have been reported (Hue et al., 2015), reinforcing the need for development of new alternatives for control of cattle tick, such the use of essential oils (Pazinatto et al., 2014). A study conducted by Chagas et al. (2002) demonstrated that essential oil of eucalypt exerted an important acaricide effect, as well as it had higher repellent and insecticide effects on *Culex pipiens* and *Musca domestica* (Batish et al., 2008). According to Olivo et al. (2013), the use of essential oil of eucalypt has some limitations, such as low aqueous solubility, volatilization and instability, in different conditions. Thus, nanotechnology can be considered an interesting approach in order to provide protective effect on the active principle and, consequently, potentiating its effects. Therefore, the aim of this study was to evaluate whether nanosystems (nanoemulsions and nanocapsules), containing essential oil of eucalypt, can interferes on the reproduction of *R. microplus,* potentiating its carrapaticide effects on the cattle tick as a natural treatment.

MATERIAL AND METHODS

Ticks

More than 330 ticks were obtained from cows naturally infected, raised in a dairy farm, located in the town of Quilombo, State of Santa Catarina, Brazil. Engorged females ticks, with average weight of 0.22g, were stored in plastic bottles and kept at 13 **°C** in Styrofoam boxes, and immediately transported to the laboratory to initiate testing.

Eucalyptus globulus essential oil

The essential oil was commercially obtained from Ferquima Ltda (Vargem Grande Paulista, São Paulo, SP, Brazil), and was subjected to the characterization (Godoi et al., 2017). The gas chromatography (GC) analyses was performed with a gas chromatograph Varian one Star 3400CX (CA, USA) equipped with a flame ionization detector (GC-FID).

The constituents of essential oil of *E. globulus* were described by Godoi et al. (2017). Twelve components, representing 100% of total composition, were identified. The results indicated that 1,8-cineole (75.78%) was the most abundant compound, followed by p-cymene (7.55%) and alpha-pinene (7.39%).

Preparation of nanoemulsion and nanoparticles containing the essential oil of *E. globulus*

The nanoemulsion and nanoparticles were prepared, having a final concentration of 5% of essential oil of *E. globulus*. The nanoemulsion was prepared according the method of emulsification under high agitation using Ultra Turrax®, as described by Godoi et al. (2017). The oil phase was composed by *E. globulus* essential oil and sorbitan monooleate (Span 80), while the aqueous phase was composed by polysorbate 80 (Twenn 80) and 25 mL of ultra-pure water. Both phases were previously solubilized using magnetic stirrer during 15 min. After, the oil phase was injected in the aqueous phase at agitation of 10.000 and 17.000 RPM during 1 h.

The nanocapsules were prepared using the method of deposition of pre-formed polymer according the methodology described by Fessi et al. (1989). The oil phase was composed by acetone, polycaprolactone (PCL), sorbitan monooleate (Span 80) and eucalypt oil, while the aqueous phase was composed by ultra-pure water and polysorbate 80 (Tween 80). After solubilization, the oil phase was injected in the aqueous phase and maintained in agitation during 10 min. Posteriorly, the solution was rotaevapored to obtain a final volume of 25 mL.

The pH determination for the nanosystems were performed using a potentiometer (Digimed®), previously calibrated with buffer solutions at pH 4.0 and 7.0. The determination of the diameter and polydispersity of nanoparticles in suspension were performed by dynamic light scattering using the Zetasizer®, Nano-ZS from Malvern equipment. The nanosystems were diluted 500 times (v/v) in Milli-Q water and the results were determined by the average of three replicates. The zeta potential was obtained using the technique of electrophoretic mobility Zetasizer®, Malvern Nano-ZS instrument. The samples were pre-diluted at 500 times (v/v) in 10 mM sodium chloride and filtered through a membrane with 0.45 micrometers. The results were expressed in millivolts (mV).

Physical and chemical properties of nanoemulsions (NE) and nanocapsules (NC) containing Eucalyptus globulus essential oil regarding size (NE 69 ± 0.71; NC 162.1 ± 1.25*), polydispersity (NE* 0.20 ± 0.01; NC 0.165 ± 0.01*), potential zeta (NE* -16.5 ± 0.65; NC -14.3 ± 0.60*) and pH (NE* 4.68 ± 0.04; NC 4.71 ± 0.06*), as previous describe by Godoi* et al. *(2017).*

Bioassays

Eucalyptus globulus, at its pure form, was tested at concentrations of 1, 5 and 10%, while formulations of *E. globulus* in nanocapsules and nanoemulsions were tested at concentrations of 0.5, 1 and 5%. Ten ingurgitated females ticks were placed in *Petri* dishes. Then, ticks were submersed in solutions containing pure oil, nanoemulsion and nanocapsules, during 30s, at concentrations described previously. Later, ticks were dried in paper towel and placed again in *Petri* dishes, stocked during 14 days B.O.D. oven, at 27 ºC, 75% RU. After 14 days, the number of ovipostures (partial or total) and the weigh eggs per dishes were evaluated.

These eggs were maintained in B.O.D. during 35 days for the percentage of larvae hatchability evaluation. By using the number of oviposture (partial or total), weight eggs and rate of hatched larvae (Drummond et al., 1973; Camillo et al., 2009), it was determined the reproductive efficiency and treatment efficacy, based in the mathematic model proposed by Camillo et al. (2009).

Statistical analysis

Normality test was applied, showing normal distribution data. Then, these data were statistically analyzed (ANOVA) and Duncan test, being considered significant when P<0.05.

RESULTS

The full results obtained are shown in the **Table I**. The pure oil of *E. globulus*, at concentration of 10%, reduced in 60% the number of ticks that performed oviposture. However, the nanosystems, containing essential oil of eucalypt, did not significantly reduced the number of ticks that performed oviposture (P>0.05), when compared with the control group. As a consequence, only the treatment with pure oil (at 5 and 10%) was able to reduce the weight of eggs, an effect not observed in the treatments with nanostructured forms. The hatching rate decreased in the treatment with pure oil (at 1, 5 and 10%), while the treatments with nanostructured forms were not able to decrease the hatching rate.

Results of reproductive efficiency and treatment efficacy, using pure oil or it in its nanostructured forms are shown in **Table II**. In summary, the pure oil (at 5 and 10%) presented 85 and 97% of efficacy. However, its nanoemulsion and nanocapsules presented 61.2 and 50% of efficacy, at 5% concentration, respectively.

DISCUSSION

Our results demonstrated that essential oil of eucalypt, in its pure form, decreases the reproductive efficiency of female ticks, obtaining excellent efficacy at concentration of 10%. However, nanoemulsion and nanocapsules, containing essential oil of *E. globulus* was not effective in controlling cattle tick. These results might are explained due the short exposure time (30s) of the ectoparasites to nanostructures, since the oil needs a reasonable time to be released (Volpato et al., 2015). In attempt to improve the efficacy of nanostructures, we believe that by increasing oil concentration may is an alternative to be considered to solve this issue. It is important to emphasize that nanotechnology uses reduced particles, facilitating the penetration of oil in the ticks; thus, increasing its biodisponibility. Unfortunately, it not occurred in this study.

Several studies reported that essential oils exert potent carrapaticide effect [basil oil (Santos et al., 2012), neem oil (Broglio-Micheletti et al., 2010) melaleuca oil (Pazinatto et al., 2014), copaiba and andiroba oils (Volpato et al., 2015), and lemongrass, cedar and rose palm oils (Pazinatto et al., 2016). The effects of oils on ticks can be considered an isolate effect of unique

Table I. **Mean and standard deviation of the number of ticks (*Rhipicephalus* (*Boophilus*) *microplus*), egg weight and hatching eggs after each treatment with essential oil of *Eucalyptus globulus* in its pure (EO) and nanostructured (EO nanoemulsion, EO nanoparticles) forms (Media y desviación estándar del número de garrapatas** (Rhipicephalus (Boophilus) microplus), peso del huevo y huevos eclosionados después de cada tratamiento con aceite esencial de ***Eucalyptus globulu***s en sus formas puras (EO) y nanoestructuradas (EO nanoemulsión, EO nanopartículas).

Treatment	Number posture by treatment (n=10)	Weighing eggs per treatment (g)	Eggs hatch (%)
Control (untreated)	10.0 ± 0.0[a]	0.59 ± 0.029[a]	80.0 ± 5.0[a]
Control (10% triton*)	9.25 ± 0.56[ab]	0.47 ± 0.071[a]	82.5 ± 5.6[a]
Control (5% nanoemulsion#)	9.75 ± 0.35[ab]	0.54 ± 0.057[a]	76.4 ± 7.5[ab]
Control (5% nanocapsules+)	9.25 ± 0.55[ab]	0.46 ± 0.035[a]	83.4 ± 10.5[a]
EO oil (1%)	8.5 ± 0.45[b]	0.40 ± 0.116[a]	63.0 ± 6.5[cd]
EO oil (5%)	8.2± 0.34[b]	0.24 ± 0.028[b]	33.4 ± 8.0[e]
EO oil (10%)	4.0 ± 0.62[c]	0.10 ± 0.011[c]	11.8 ± 2.2[f]
EO nanoemulsion (0.5%)	10.0 ± 0.0[a]	0.51 ± 0.062[a]	60.2 ± 5.5[d]
EO nanoemulsion (1%)	9.25± 0.64[ab]	0.48 ± 0.035[a]	62.4 ± 4.6[cd]
EO nanoemulsion (5%)	8.5 ± 0.41[b]	0.40 ± 0.165[a]	70.0 ± 8.0[bc]
EO nanocápsulas (0.5%)	9.25 ± 0.30[ab]	0.49 ± 0.025[a]	85.0 ± 4.0[a]
EO nanocápsulas (1%)	9.0 ± 0.20[ab]	0.53 ± 0.018[a]	77.5 ± 9.5[ab]
EO nanocápsulas (5%)	8.5 ± 0.35[b]	0.50 ± 0.030[a]	80.0 ± 5.0[a]

Mean followed by equal letter in the same column did not differ among treatments at significance of 5% in the Duncan test. *Triton was used as diluents of essential oil of *Eucalyptus globulus* (1v/v). # White nanoemulsion was formed by sorbitan monooleate (Span 80) and polysorbate 80 (Twenn 80). +White nanocapsules was formed by cetyl palmitate and polysorbate 80.

Table II. Degree of efficacy on reproduction and treatment with essential oil of *Eucalyptus globulus* in its pure (EO) and nanostructured (EO nanoemulsion and EO nanoparticles) form (Grado de eficacia sobre la reproducción y tratamiento con aceite esencial de *Eucalyptus globulus* en sus formas puras (EO) y nanoestructuradas (EO nanoemulsión y nanopartículas de EO).

Treatment	Reproductive efficiency (%)	Efficacy of treatment (%)
Control (untreated)	53.9[a]	0.0[g]
Control (10% triton)	50.6[a]	5.5[f]
Control (5% nanoemulsion)	52.6[a]	3.8[fg]
Control (5% nanocapsules)	49.9[a]	6.7[f]
EO oil (1%)	22.9[d]	59.3[c]
EO oil (5%)	8.4[e]	85.0[b]
EO oil (10%)	12.0[e]	97.8[a]
EO nanoemulsion (0.5%)	29.7[c]	47.2[d]
EO nanoemulsion (1%)	31.0[c]	50.0[d]
EO nanoemulsion (5%)	27.9[cd]	61.2[c]
EO nanoparticles (0.5%)	38.4[b]	31.0[e]
EO nanoparticles (1%)	38.7[b]	31.3[e]
EO nanoparticles (5%)	36.0[bc]	35.5[e]

Mean followed by equal letter in the same column did not differ among treatments at significance of 5% in the Duncan test. Reproductive efficiency and efficacy of the treatment is detailed in Drummond et al. (1973) and Camillo et al. (2009).

component; either can be considered an association among components present in the oil (Walton et al., 2014; Pazinatto et al., 2016). The 1,8-cineole, is the most abundant compound present in eucalypt essential oil, exerting repellent effect on *Ixodes ricinius* (Acari: Ixodide) (Ashitani et al., 2015) and insecticidal and repellent effects on *Aedes aegypti* (Dias and Morais 2014). These are similar effects as the observed in our study.

Based on these results, we concluded that use of nanotechnology, producing nanoemulsion and nanocapsule containing essential oil of *E. globulus*, showed low efficacy on *R. microplus*. Therefore, the nanotechnology would not be considered an option to potentiate the effects of pure oil, as tested in our experimental model. However, we cannot rule out the possibility that other species of eucalypt may have their effects potentiated after association with nanocarriers, or even its use in higher concentrations.

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