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FREE ESSENTIAL OILS AND NANOSTRUCTURED ON *TRICHOMONAS GALLINAE* TROPHOZOITES[1]

ÓLEOS ESSENCIAIS LIVRES E NANOSTRUTURADOS EM TROFOZOITOS DE TRICHOMONAS GALLINAE[1]

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ABSTRACT

Avian trichomoniasis mainly affects the upper digestive tract of birds and it is caused by the flagellate protozoan, *Trichomonas gallinae*. The present study was developed to evaluate the *in vitro* effects of free essential oils (OEs) and nanostructured *Ocimum basilicum* and *Eucalyptus globulus* against trophozoites of *T. gallinae*. *T. gallinae* trophozoites were recovered by the swab method from naturally infected urban pigeons, followed by cultivation in TYM medium (trypticase-yeast-maltose extract). The *in vitro* assays were performed in 96-well plates containing essential oil (OE) and nanostructured on different concentrations in medium containing $1x10^6$ parasites. Four controls were performed: NC (culture medium and trophozoites), MTZ (trophozoites plus 25 µg / ml metronidazole), TW (trophozoites plus vehicle for solubilization of derivatives (0,1% Tween) and NB (blank nanoemulsion 1%). To verify the viability of trophozoites, motility, morphology, and exclusion by trypan blue dye (0.4%) were observed. The analysis of the data obtained from the screening of the compounds showed that 1,5% EO of *O. basilicum* and 1,25 of nanoemulsion reduced the viability of trophozoites by 100%, relative to the negative control. OE *E. globulus* 1,5% and 2% nanoemulsion. The controls CN, TW and NB exhibited positive motility and negative staining with tripan blue, while control MTZ reduced the viability of the parasite by 100% after 24-hour exposure. The data obtained in this study indicate that OEs and nanoemulsions may contribute as effective agents in the control of *T. gallinae* infections.

Keywords: birds, nanotechnology, phytotherapy, protozoa.

RESUMO

A tricomoníase aviária afeta principalmente o trato digestivo superior das aves, causado pelo protozoário flagelado Trichomonas gallinae. O presente estudo foi desenvolvido para avaliar os efeitos in vitro de óleos essenciais livres (OEs) e nanoestruturados de Ocimum basilicum e Eucalyptus globulus, contra trofozoítos de T. gallinae. Os trofozoítos de T. gallinae foram recuperados pelo método do swab de pombos urbanos naturalmente infectados, seguidos pelo cultivo em meio TYM (extrato de tripticase-levedura-maltose). Os ensaios in vitro foram realizados em placas de 96 poços contendo óleo essencial (OE) e nanoestruturados em diferentes concentrações em meio contendo $1x10^6$ *parasitos. Foram realizados quatro controles: NC (meio de*

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cultura e trofozoítos), MTZ (trofozoítos mais 25 µg / ml de metronidazol), TW (trofozoítos mais veículo para solubilização de derivados (0,1% Tween) e NB (nanoemulsão branca a 1%). Para verificar a viabilidade dos trofozoítos, motilidade, morfologia, pela exclusão pelo corante azul de tripan (0,4%). A análise dos dados obtidos na triagem dos compostos mostrou que 1,5% de OE de O. basilicum e 1,25 de nanoemulsão reduziu em 100% a viabilidade dos trofozoítos em relação ao controle negativo. OE E. globulus nanoemulsão 1,5% e 2 %. Os controles CN, TW e NB exibiram motilidade positiva e coloração negativa com azul de tripan, enquanto o controle MTZ reduziu a viabilidade do parasito em 100% após 24 horas de exposição. Os dados obtidos neste estudo indicam que os OEs e as nanoemulsões podem contribuir como agentes efetivos no controle das infecções por T. gallinae.

Palavras-chave: *aves, fitoterapia, nanotecnologia, protozoários.*

INTRODUCTION

Avian trichomoniasis is a disease caused by the flagellate protozoan *Trichomonas gallinae*, which is the most affected species belonging to the family Columbidae (BRANDÃO; BEAUFRÈRE, 2013; PURPLE;GERHOLD, 2015). A protozoan infects wild and captive bird species worldwide and migrating birds may contribute to its spread (ROBINSON *et al*., 2010). Due to its widespread occurrence and sometimes its pathogenic character, it is believed that *T. gallinae* plays a role in the regulation of wild bird populations (ROBINSON *et al*., 2010; STABLER, 1954; KRONE *et al*., 2005; FORRESTER;FOSTER, 2009; AMIN *et al*., 2014). Birds of prey may develop trichomoniasis by feeding on infected prey, particularly pigeons (*Columba livia*), main hosts of *T. gallinae* (BUNBURY *et al*., 2008a), causing mortality of adults and pups (KRONE *et al*., 2005; AMIN *et al*., 2012; BUNBURY *et al*., 2008b; STOCKDALE *et al*., 2015). Transmission may also occur at shared feeding sites among several bird species, but the main form is during feeding of pups by infected parents (STABLER, 1954; KOCAN, 1969).

Drugs for the treatment of trichomoniasis are nitroimidazoles. However, the use of subdoses and the preventive use of these drugs against avian trichomoniasis have resulted in the emergence of resistant strains (LUMEJI;ZWIJNENBERG, 1990). Despite the existence of nitroimidazole-resistant strains of *T. gallinae*, very little research has been done with alternative means of treatment.

One of the alternatives is the use of essential oils (OE), which are used due to their chemical compounds and have antibacterial, antifungal, antiviral, antiprotozoal and anti-inflammatory activities (CARSON *et al*., 2006; HAMMER *et al*., 2008).

Besides the use of OEs, nanotechnology has been used for the development of nanostructured systems that can increase the effectiveness of the oil, mainly raising its stability and the contact time on the target tissues. In our study, nanostructured OEs appear as a new alternative for the treatment in search of a new molecule of this disease, since these systems can, through their high specific surface and reduced surface tension, promote greater penetration of active substances at specific sites

(SALAMANCA-BUENTELLO *et al*., 2005; SILVA, 2004), have less impact to the host, as well as improve the efficacy in the treatment of avian trichomoniasis.

The objective of the present study was to evaluate the efficacy of essential oils and nanoemulsions of *Eucalyptus globulus* (eucalyptus) and *Ocimum basilicum* (basil), besides also evaluating new non-cytotoxic and pharmacologically active compounds *in vitro* against trophozoites of *T. gallinae*.

MATERIAL AND METHODS

REAGENTS

OE basil OE (*O. basilicum*) and metronidazole used as control in the *in vitro* test were purchased from Sigma-Aldrich®, Germany. Eucalyptus (*E. globulus*) of FERQUIMA Indústria e Comércio Ltda.

DEVELOPMENT AND CHARACTERIZATION OF NANOEMULSIONS

The essential oils and nanoemulsions in *O. basilicum* and *E. globulus* were supplied by the Nanotechnology Laboratory of the Universidad Fransciscana (UFN). The characterization of essential oils allowed the method described by Hussain *et al*., 2010 with modifications. Using the Varian Star 3400CX gas chromatograph (CA, USA). For the qualitative analysis of the compounds, a Shimadzu QP2010 Plus gas chromatograph coupled to a mass spectrometer (GC / MS, Shimadzu Corporation, Kyoto, Japan) was used (GODOI *et al*., 2017; GÜNDEL *et al*., 2018).

DEVELOPMENT OF NANOEMULSIONS

The nanoemulsions containing Basil (*O. basilicum*) or Eucalyptus (*E. globulus*) oil were developed using the homogenization under high agitation method, following the methodologies described by Gündel *et al*. 2018 and Godoi *et al*., 2017 , respectively. The formulations were composed of an oil phase containing essential oil (5%) eucalyptus or 7.5% basil and sorbitan monooleate surfactant (2%), while the aqueous phase was composed of polysorbate 80 (2%) and ultrapure water. Both phases were homogenized separately with the aid of a magnetic stirrer, then the aqueous phase was placed in the Ultra-Turrax® (IKA, Germany) equipment for 10 minutes at 10,000 rpm. Subsequently, the oil phase was injected into the aqueous phase, and maintained in the Ultra-Turrax® for 30 minutes at 17,000 rpm, with temperature control. The blank nanoemulsion was developed using a medium chain triglyceride, derived from caprylic and capric acids (GODOI *et al*, 2017; GÜNDEL *et al*., 2018).

CHARACTERIZATION OF NANOEMULSIONS

The physicochemical characterization of the formulations was evaluated by determining the mean droplet size, polydispersity index, zeta potential and pH. The mean droplet size and polydispersity index were determined by the dynamic light scattering technique (Zetasizer® equipment, nano-ZS model ZEN 3600, Malvern) after sample dilution (500 times) in ultrapure water. The zeta potential was determined using the electrophoretic mobility technique (Zetasizer® equipment, nano-ZS model ZEN 3600, Malvern) after sample dilution (500 times) in aqueous solution of sodium chloride (10 mM). The pH was determined using a potentiometer (DM-22, Digimed®) previously calibrated with standard solution, and the readings were carried out directly in formulations. All formulations of the nanoemulsions remained stable under refrigeration for up to 90 days. The readings were done in triplicate and the results expressed as mean ± standard deviation (GODOI *et al*., 2017; GÜNDEL *et al*., 2018).

TRICHOMONAS GALLINAE

Specimens of *T. gallinae* were recovered by the wet mount method of naturally infected pigeons. Twelve native pigeons (2 to 8 weeks old) were captured in their nests in the city of Pelotas, Rio Grande do Sul. This research was approved by the Committee of Ethics in the use of animals of the Federal University of Pelotas, 05/07th/2018, under the protocol 23110.012860 / 2018-81 and SISBIO on 02/08th/2018, under number 61235-1.

Using swabs, samples were taken from the oral cavity, and from membranous lesions of the oropharyngeal region of domestic pigeons (*C. livia*). The culture of the parasite was prepared by immersion of oral swabs in tryptone / yeast extract / maltose medium (TYM) Diamond, (1957), supplemented with 10% adult fetal serum, antibiotic (meropenem), antifungal (amphotericin B) and incubated at 37 ° C (SANSANO *et al*., 2009). The cultures were then examined under an optical microscope at (100 and 400 x) for observation of mobile *T. gallinae* trophozoites.

Cultures were observed during seven consecutive days to verify the growth of trophozoites. Every 48 hours, such as trophozoites that had more than 95% mobility and normal morphology, they were subcultured (SEDDIEK *et al*., 2014).

IN VITRO ASSAY

To examine the susceptibility of *T. gallinae* to essential oils and nanoemulsions, sterile 96-well plates were used for incubation with different concentrations of essential oil and nanoemulsion. The nanoemulsions used for the *in vitro* assay were applied after seven days of their formulations preparation.

The parasites were seeded at an initial density of 1x106 trophozoites / mL of TYM and incubated with the oils and nanostructured. Three controls were performed: A (trophozoites only), B (trophozoites plus the vehicle for solubilization of the derivatives (0.01% Tween) C (trophozoites plus 100mM metronidazole) (as positive control, and D (blank nanoemulsion).

The essential oils and nanoemulsions were added to the wells to obtain final concentrations of 0,25 and 2%, respectively. Subsequently, to generate anaerobic conditions, the microculture plates were incubated at 37°C with 5% CO2 for 24 h. After this period, a preparation with trophozoites, containing tripan blue (0.4%) (1:1), was evaluated in Neubauer chamber. Cultures with viability equal or greater than 95% were used for assays, being considered motility, morphology and exclusion by death.

The IC50 (half of the maximum inhibitory concentration) was determined at concentrations for the oils and nanoemulsions. A kinetic growth curve was constructed to obtain the profile of comparable activity of compounds OEs and nanoemulsions against *T. gallinae* trophozoites.

Only the OEs and nanoemulsions that showed a reduction in the viability of the trophozoites to 100% were used to determine the MIC and were used under different concentrations. The trophozoites used to establish MIC and low and high concentrations, as well as controls inoculated in fresh TYM medium at 37°C, were counted in Neubauer chamber with tripan blue every 24 hours for 96 hours to confirm MIC.

The best concentrations, presented after MIC analysis for each essential oil and nanoemulsion, were performed at the following times: 1, 6, 12, 24, 48, 72, and 96 hours by the death exclusion method. The IC50 was determined at varying concentrations, as described in the MIC method. A death time curve was constructed to obtain an activity profile of the efficacy of essential oil and nanoemulsion against *T. gallinae* flagellate trophozoites. All assays were performed independently in nonoplicata (SENA-LOPES *et al*., 2017).

ANALYZE STATISTICAL

Statistical analysis was performed by univariate analysis of variance (ANOVA) using a probability value of $p \leq 0.05$ followed by the Tukey Test (GraphPad Prism 8.0 Software).

RESULTS

CHARACTERIZATION ESSENTIAL OIL

The OE components of eucalyptus and basil were analyzed by gas chromatography. The major compounds of the *E. globulus* EO and its main components were 1,8-cineole (75.7%); p-cymene (7.5%); alpha-pinene (7.3%); and limonene (6.4%). In the analysis of the basil oil, the estragol was identified as the main constituent, which represented about 87%, in the sequence are the compounds 1,8-cineol and trans-alpha-bergamotene presenting values of normalization around 3 and 2%, respectively (GODOI *et al*, 2017; GÜNDEL *et al*., 2018).

CHARACTERIZATION OF NANOEMULSIONS

All nanoemulsions presented a polydispersity index lower than 0.3, indicating homogeneity of droplet size and characterizing a monodisperse system (ADUKU *et al*., 2016; XAVIER *et al*., 2016). Despite the use of non-ionic surfactants in the formulation, the nanoemulsions have slightly negative zeta potential, which may be associated with the chemical properties of the polyoxyethylene chains present in the sorbitan monooleate and polysorbate 80 (MARUNO;ROCHA-FILHO, 2010). While acid pH values are related to the properties of essential oils (FLORES *et al*., 2011).

Nanoemulsions are formed by mixing two immiscible liquids stabilized by surfactants, ranging in size from 20μm to 500nm. The nanoemulsions developed in the present study had an average droplet size of less than 200 nm. The results of the characterization of nanoemulsions with OE and white nanoemulsion, according to mean size, polydisperation index, zeta potential and pH, are shown in table 1.

Table 1 - Physical-chemical characterization of nanoemulsions.

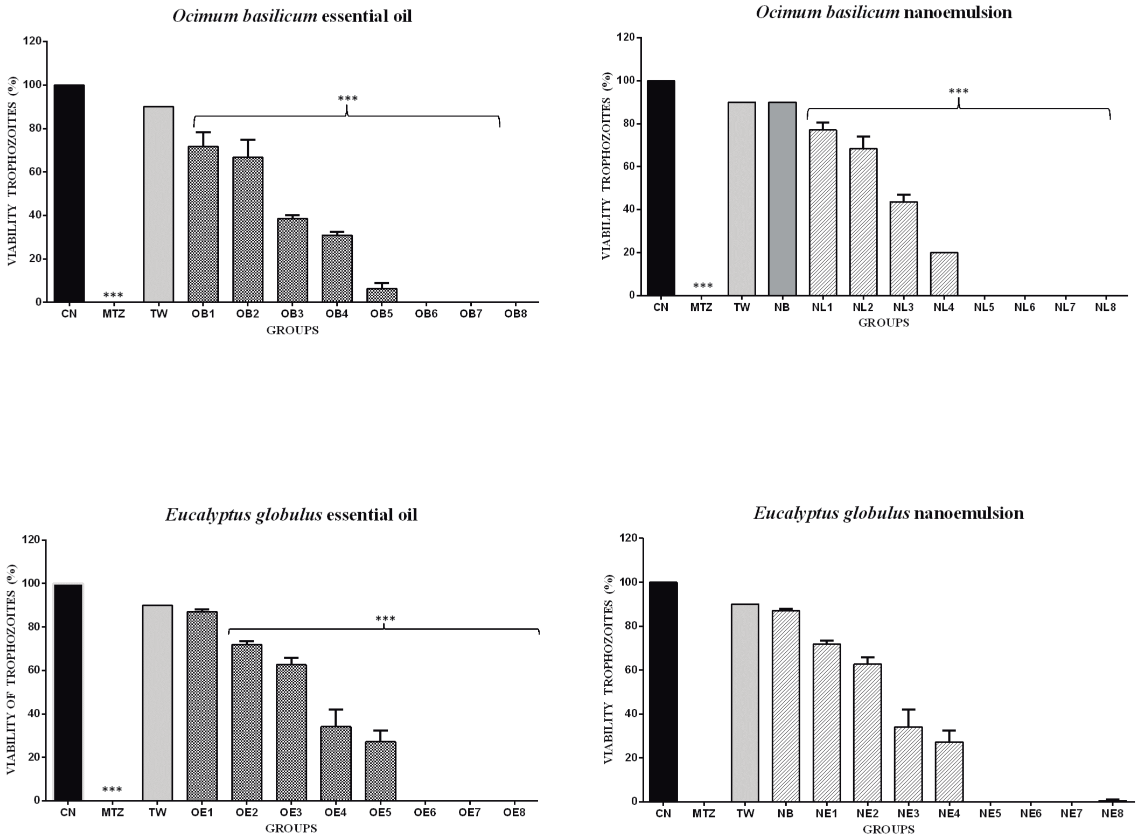
	Average droplet size (nm)	Polydispersity index	Zeta potential (mV)	pH
Nanoemulsion containing eucalyptus oil	66 ± 0.81	0.23 ± 0.004	- 7.53 ± 0.72	5.90 ± 0.04
Nanoemulsion containing basil oil	113 ± 0.78	0.19 ± 0.007	- 8.47 ± 0.98	5.37 ± 0.04
Blank nanoemulsion	127 ± 1.02	0.25 ± 0.005	- 6.37 ± 0.58	5.15 ± 0.03

The under refrigeration nanoemulsions were stable for 90 days as the parameters were maintained when compared to the parameters immediately after preparation (GODOI *et al*, 2017; GÜNDEL *et al*., 2018).

IN VITRO ASSAY

The results of the *in vitro* study on the anti-trichomonal *T. gallinae* inhibitory effect of OEs and nanoemulsions and the comparison of this activity with the drug metronidazole are presented in Table 1.

Table 1 - Anti-trichomonas activity in culture media of the formulations: essential oils and nanoemulsions in *Ocimum basilicum* and *Eucaliptus globulus* at concentrations of 0,25- 2%, as controls: CN (negative control), MTZ (metronidazole), TW (tween 80%) and NB (Blank nanoemulsion). The analyzes were evaluated at 24 hours post-treatment. The columns indicate the groups and * indicate the statistical difference when compared to the MTZ control by Tukey's test (P <0.05).



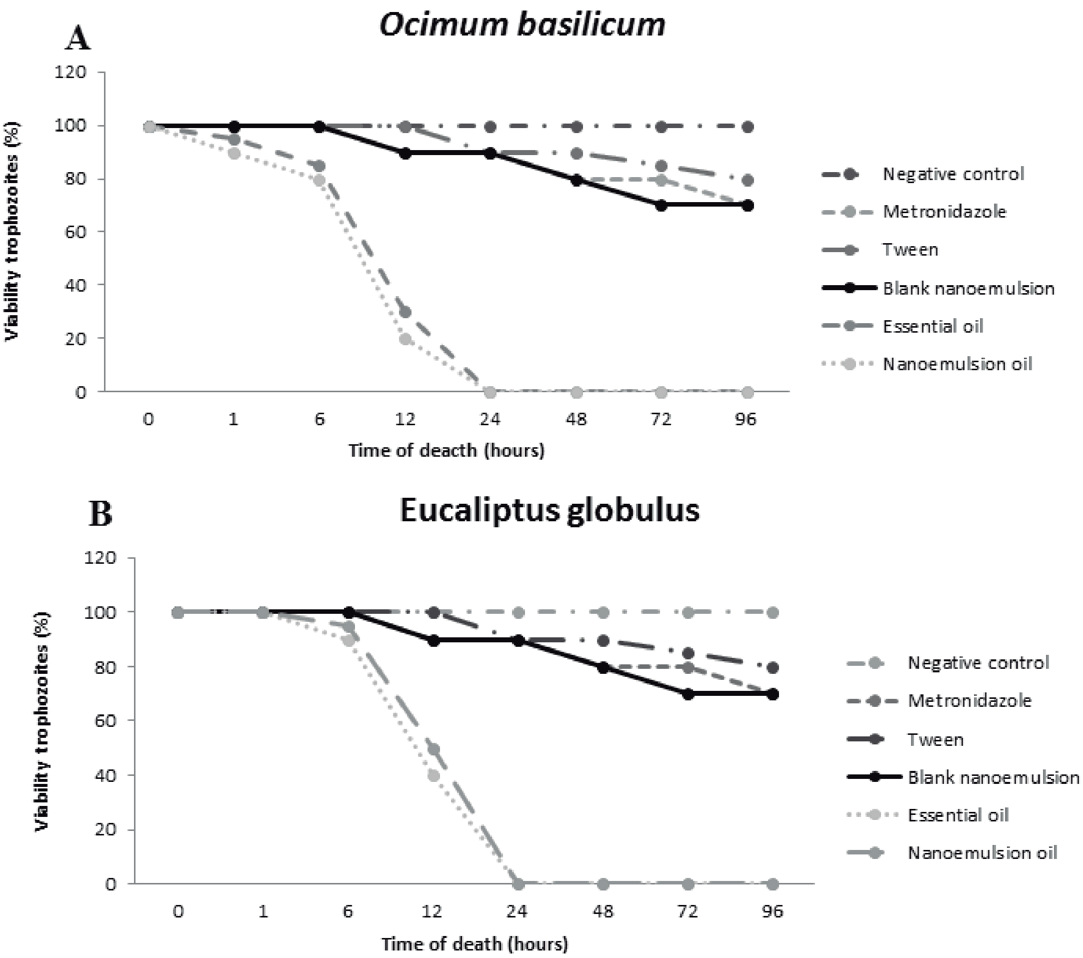
Source: Bruna Baccega

The essential oil and nanoemulsion of the compounds tested against *T. gallinae*, with emphasis on basil, showed efficiency at lower concentrations when compared to eucalyptus. For basil (*O. basilicum*), after 24- hour incubation at 1,25% concentration for nanoemulsion and 1.5% for essential oil, 100% unviable trophozoites in the culture medium. The MIC (minimum inhibitory concentration) of the essential oil and the nanoemulsion were 1,45% and 1,20%. IC_{50} values (mean inhibitory concentration) were set at 0,5% for nanoemulsion and 0,75% for essential oil.

The essential oil and nanoemulsion of eucalyptus (*E. globulus*) showed inhibitory activity at the concentration of 1,5% and 2%, revealing its effectiveness in 24 hours, with no significant difference between them (Fig. 1). The MIC was 1,45% and 1,90% and the IC50 was 0,75% and 1%, respectively.

The growth of the trophozoites was completely inhibited by the essential oils and nanoemulsions of basil and eucalyptus, the inhibition of 100% of the trophozoites after 24-hour incubation (Table 2).

Table 2 - Time of death of *Trichomonas gallinae* after treatment with negative control group, metronidazole, tween 80, blank nanoemulsion, essential oil and nanoemulsion basil and eucalyptus, in the period of 0, 1, 6, 12, 24, 48, 72 and 96 h.



Source: Bruna Baccega

DISCUSSION

The study of the use of herbal medicines has increased significantly in recent years. The use of medicinal plants with potential effects and phytotherapics has been increasing for the treatment of trichomoniasis, mainly for the species *Trichomonas vaginalis*. Iran has already been developing studies of native plants (IBRAIM, 2013), but in Brazil this study represents unpublished results with phytotherapy for the *T. gallinae* protozoan.

The emergence of resistance to the drugs used for the treatment of avian trichomoniasis has been reported in different parts of the world, as in studies by Lumeij and Zwijnenberg (1990) in Russia; Franssen and Lumeij (1992) in Europe; Gerhold *et al*. (2008) in Belgium, Munoz *et al*. (1998) in Spain, Rouffaer *et al*. (2014) The United States and Tabari *et al*. (2017) in Iran. Resistant strains end up becoming a threat to bird sanity (FORRESTER;FOSTER, 2009) and the parasite is found in species, such as: pigeon, sparrow, cardinal, quail, parrot, domestic birds, chicken, turkey, not infrequently causing economic losses (BONDURANT;HONIGBERG, 1994).

Resistances to current chemotherapies evidenced the need for new antiparasitic agents, such as the use of OEs and plant nanoemulsions (SOLÓRZANO-SANTOS *et al*., 2011, BARROS *et al*., 2016). There are reports of resistant isolates of *T. gallinae* to nitroimidazole, and their prevalence reaches 80-100%, few studies have been conducted to find alternative trichomonicidal agents (HARLIN*;*WADE, 2009). The present work is a pioneer in studies related to essential oil and nanoemulsions on the flagellate protozoan *T. gallinae* in Brazil, with promising results for OE basil nanoemulsion.

Treatment with basil, eucaliptus and metronidazole resulted in no viable trophozoite in the culture medium after 24 hours, corroborating with the data from Youssefi *et al*. (2017) and Tabari *et al*. (2017). These essential oils demonstrated the antitrichomonal effects against trophozoites *T. gallinae*, at the assay *in vitro*. Several drugs were tested active trichomoniasis in pigeons (AYDIN*;*COŞKUN 2000; EL-SAYED 2005), but the potential risks of carcinogenicity (SOBEL *et al*., 2010) and drug resistance (FRANSSEN*;*LUMEIJ 1992) against MTZ made the development of a new antitrichomonal drug an urgent need. Since ancient times, various herbal medicines have been tested to treat different parasites (KANOJIYA *et al*., 2015).

The EO and nanoemulsions used in the present study, at their respective concentrations, have already been tested under other microorganisms, without cytotoxicity (GODOI *et al*., 2017; GÜNDEL *et al*., 2018).

The three chemical constituents predominant in these OEs in this study were previously identified as major constituents, however, at different concentrations (VITTI*;* BRITO, 2003; MARTINEZ-VELAZQUEZ *et al*., 2011, MOKHTARZADEH *et al*., 2017). Variations in chemical constituents occur due to several factors: seasonality, temperature, collection, availability of water, among others (XAVIER *et al*., 2016). In our data the chemical constituents of the OEs tested showed activity against the flagellated *T. gallinae* protozoan, at different concentrations, inactivating the same in the 24-hour period. The activity of basil OEs is related to bioactive components that have estragol. Its activity is mainly associated with one of its component, estragol, which has already exhibited activity in other studies, such as the *Eimeria tenella* (JITVIRIYANON *et al*., 2016), *G. lamblia* (BARBOSA *et al*., 2007), *T. gallinae* (ADEBAJO *et al*., 2006), *T. vaginalis* (ARTHAN *et al*., 2008), and *Leishmania infantum* (GONZALEZ-COLOMA *et al*., 2011). In our study, basil oil showed higher efficacy at shorter time when compared to essential oil of eucalyptus. It has been demonstrated essential oil has the ability to destroy the integrity of the cell membrane, releasing the cellular components and causing morphological changes in the plasma membrane, which may cause damage to the organelles, and thus, cellular lysis of the trophozoites (BOYOM *et al*., 2003).

The efficacy of basil nanoemulsion, when compared to essential oil and nanoemulsion in eucalyptus, had a greater efficacy ($P < 0.05$). This fact can be attributed to the chemical compounds present in this essential oil, particle size reduction and greater bioavailability. The relevance of researches with nanostructured systems with bioactive withdrawn from plants is evident as seen in the works of

Schmaltz *et al*. (2005), Codevila *et al*. (2015), Bahamond *et al*. (2015) and Wen-Chien *et al*. (2018). The essential oils of basil and eucalyptus presented anti-*trichomonas* activity, both in their free formulation, as well as, nanostructured. However, the basil formulation evidenced its strongly potentiated activity, since it caused 100% mortality of the trophozoites in a lower time interval when compared to the OEs tested.

Tabari *et al*. (2017) suggests that *P. harmala* is a natural antitricomonal agent effective against *T. gallinae* when compared to metronidazole, with a similar result to that of the present study, when compared to essential oil and nanoemulsion of basil at concentrations of 1,25% and 1,5%. Nikpay and Soltani (2018) evaluated the anti-trichomonas activity of the extracts of *Pulicaria dysenterica* and *Lycopus europaeus* at different concentrations. The results showed that both extracts decreased viability in 10% and 60% inhibition of the growth of the culture, results inferior to those obtained in the present work, in which the minimum inhibitory concentration (MIC) was 100% mortality of the trophozoites.

The essential oil *O. basilicum* had lethal effects on the growth of the trophozoites, with ultrastructure destructive changes. In this study the effect of the *O. bacilicum* oil on the inhibition of trophozoites growth showed statistically significant difference when compared to parasite negative control, inhibition of trophozoites growth, and trophozoites motility were in relation to concentration and incubation in 24-hours time. These findings were in accordance with the results of *T. gallinae* (ADEBAJO *et al*. 2006) and *T. vaginalis* (ARTHAN *et al*. 2008; ELDIN *et al*., 2015). It is believed that the inhibitory effect of *O. basilicum* oil on the growth of trophozoites is, also possibly, through its ability to inhibit activity of cysteine proteinase of *T. gallinae*, as claim Gradoni and Ascenzi (2004) under the protozoa *T. vaginalis*.

The eucalyptus EO has therapeutic application and Silva *et al*. (2003), Batish *et al*. (2008), Matos *et al*. (2004) and Chagas *et al*. (2002) state that some species of eucalyptus have activity against protozoa. In this study, the evaluation performed with the essential oil and nanoemulsion of *Eucalyptus globulus* on MIC (1,90%), occurred the death of the trophozoites in 24-hours exposure. This data show the lower efficacy of this bioactive compound when compared to the results observed with basil OE, because the lower the MIC, the greater the potency and, consequently, the greater the difficulty of the microorganism to develop resistance.

The eucalypt EO effectiveness was higher when compared to its nanoemulsion, which has a slower release to the trophozoites, breaking the nanostructure later. These results differ from those observed by Chagas *et al*. (2002) who demonstrated biocidal action of Eucalyptus species on the *Rhipicephalus (Boophilus) microplus* tick, and observed that the essential oils had their activities potentiated when transformed into emulsifiable concentration. This eucalypt EO efficacy when compared to nanoemulsion can also be attributed to dilution of the oil that provides a slower and more effective penetration into the microorganism. Although the pure oil is lipophilic and has a lower absorption, when compared to the emulsifiable system (CHAGAS *et al*., 2002).

The use of nanoemulsions with essential oils has been gaining ground in research. Nanoemulsions can be classically defined as a nanometric dispersion of oily droplets in an external aqueous phase, stabilized by a suitable surfactant system. The oil/drug delivered is preferably dispersed or adsorbed on the oily nucleus of the nanostructure (FRONZA *et al*., 2004). In the tests carried out the nanotechnology associated with the essential oil of basil showed a high efficiency in the absorption of the target cells.

Nanotechnology associated with animal welfare has been gaining applications ranging from diagnosis to treatments in the field of pharmaceutical nanotechnology (FENEQUE, 2003). The synthesis of new synthetic molecules provides therapeutic compounds for the treatment of diseases in pets and production (CHAKRAVARTHI;BALAJI, 2010). The primary focus of research in the area of nanoemulsions is the creation of drug delivery systems. The development of these systems has made possible some improvements in the rate of absorption, distribution, metabolism and excretion of drugs or other substances in the body (FENEQUE, 2003).

The nanoemulsions used in the present study were evaluated for their stability under opposing climatic conditions. When stored at elevated temperatures (40°C), there were instability in their formulations. Stability was observed for 90 days under refrigeration, when compared to the parameters obtained shortly after preparation (GÜNDEL *et al*., 2018; SOLANS *et al*., 2005).

An emulsion when it presents a mean droplet diameter (Z) of less than 300nm is considered a nanoemulsion, corroborating with our study, in which the nanoemulsions had a diameter of less than 200 nm. Emulsions containing the smallest diameters (200-500 nm) tend to be physically more stable (MEHLHORN *et al*., 2009). The stability is due to the use of surfactants in their formulation, which increase the availability and penetration by low interfacial tension. The droplet size is a parameter of stability is relevant and of great importance (ANTON;VANDAMME, 2011; LEONG *et al*., 2009; MCCLEMENTS, 2012)

Nair *et al*. (2016) state that the therapeutic efficacy of many compounds when they are associated with nanotechnology occurs because of better solubilization, greater bioavailability and protection of the active ingredient against enzymatic and hydrolytic degradation. In our study, the results obtained in the tests with the EO of nanostructured basil demonstrate a greater efficacy. This fact could be attributed to a greater penetration of the nanoemulsion in the receptors present in the protozoan membrane, which allowed a greater interaction between them. The higher the concentration of nanoemulsion, the greater the dose-dependent efficacy, with 100% mortality occurring in the three highest concentrations tested. This result can be attributed to the size of the nanoparticles (<200 nm). Trados *et al*. (2004) affirm that nanoemulsions with the size and biocompatibility of 20-200nm present advantages like greater bioavailability, which facilitates the EO activity.

CONCLUSION

The essential oils and their respective nanoemulsions were able to reduce the viability of *T. gallinae* trophozoites that were causing cell death at different concentrations.

The use of basil OE was more effective against trophozoites of *T. gallinae* with increasing concentrations of essential oil and nanoemulsion in time of 12 hours. The efficacy of EO of basil and eucalyptus only reached 100% efficacy after 24 hours of contact.

These results aim at the possibility of using essential oils and nanoemulsions as anti-*trichomonas* agents in various concentrations and may suggest the potential of these plants for the treatment of metronidazole resistant *T. gallinae* isolates. However, more comprehensive studies are needed to research about the anti-*trichomonas* activities of essential oils and nanoemulsions under in vivo conditions.

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REFERENCES

ABD EL-RAHMAN, M. A. M. *et al*. Some Studies on Trichomoniasis of Pigeons at Qualiobia Governorate. **Egyptain Journal of Comparative Pathology and Clinical Pathology**, v. 21, n. 2, p. 123-141, 2008.

ADEBAJO, A. C. *et al*. Anti-trichomonal, biochemical and toxicological activities of methanolic extract and some carbazolealkaloids isolated from the leaves of *Murraya koenigii* growing in Nigeria. **Phytomedicine**, v. 13, n. 4, p. 246-254, 2006.

ADUKWU, E. C. *et al*. Antimicrobial activity, cytotoxicity and chemical analysis of lemongrass essential oil (*Cymbopogon flexuosus*) and pure citral. **Applied Microbiology and Biotechnology**, v. 100, n. 22, p. 9619-9627, 2016.

AMIN, A. *et al*. Trichomonads in birds - a review. **Parasitology**, v. 141, n. 5, p. 733-47, 2014.

ANTON, N.; VANDAMME, T. F. Nano-emulsions and micro-emulsions: clarifications of the critical differences. **Pharmacology Research**, v. 28, n. 5, p. 978-85, 2011.

ARTHAN, D. *et al*. Inhibitory effects of Thai plants b-glycosides on *Trichomonas vaginalis*. **Parasitology Research**, v. 103, n. 2, p. 443-448, 2008.

AYDIN, L.; COŞKUN, Ş. Efficacy of carnidazole (Spatrix®) and metronidazole (Flagyl®) against *Trichomonas gallinae* in aturally infected pigeons. **Acta Parasitology Turcica**, v. 24, n. 1, p. 65-66, 2000.

BAHAMONDE-NORAMBUENA, D. *et al*. Polymeric nanoparticles in dermocosmetic. **International Journal of Morphology**, v. 33, p. 1563-1568, 2015.

BARBOSA, E. *et al*. *In vivo* antigiardial activity of three flavonoids isolated from some medicinal plants used in Mexican traditional medicine for the treatment of diarrhea. **Journal of Ethnopharmacology**, v. 109, n. 3, p. 552–554, 2007.

BARROS, L. M. *et al*. Characterization and Trypanocidal, Leishmanicidal and Cytotoxicity Potential of *Lantana camara* L. (Verbenaceae) Essential Oil. **Molecules,** v. 21, n. 2, p. 1-9, 2016.

BATISH, D. R. *et al*. Eucalyptus Essential Oil as a Natural Pesticide. **Forest Ecology and Management**, v. 256, p. 2166-2174, 2008.

BENZIE, I. F.; STRAIN, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. **Analytical Biochemistry**, v. 239, n. 1, p. 70-6, 1996.

BONDURANT, R. H.; HONIGBERG, B. M. **Trichomonads of veterinary importance**. *In*: KREIER, J. P. (Ed.). **Parasitic Protozoa.** New York, NY, USA: Academic Press, 1994. p. 111-188.

BOYOM, F. F. *et al*. Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. **Phytochemistry**, v. 64, p. 1269-1275, 2003.

BRANDÃO, J.; BEAUFRÈRE, H. Clinical update and treatment of selected infectious gastrointestinal diseases in avian species. **Journal of Exotic Pet Medicine**, v. 22, n. 2, p. 101-117, 2013.

BUNBURY, N. *et al*. Epidemiology and conservation implications of *Trichomonas gallinae* infection in the endangered Mauritian Pink Pigeon. **Biological Conservation**, v. 141, n. 1, p. 153-161, 2008a.

BUNBURY, N. *et al*. Causes of mortality in free-living Mauritian pink pigeons Columba mayeri, 2002-2006. **Endanger Species Research**, v. 9, p. 213-220, 2008b.

CARSON, C. F. *et al*. *Melaleuca alternifolia* (Tea Tree) Oil: a review of antimicrobial and other medicinal properties. **Clinical Microbiology Reviews**, v.19, n. 1, p. 50-62, 2006.

CHAGAS, A. C. de S. *et al*. Efeito acaricida de óleos essenciais e concentrados emulsionáveis de *Eucalyptus* spp em *Boophilus microplus*. **Brazilian Journal of Veterinary Research and Animal Science**, v. 39, n. 5, p. 247-253, 2002.

CHAKRAVARTHI V. P.; BALAJI, S. N. Applications of Nanotechnology in Veterinary Medicine. **Veterinary World**, v. 1.3, n. 10, p. 477-480, 2010.

CODEVILLA, C. F. *et al*. Nanoestruturas contendo compostos bioativos extraídos de plantas. **Ciência e Natura**, v. 37, p. 142-151, 2015.

DIAMOND, L. S. The establishment of various trichomonads of animals and man in axenic cultures. **The Journal of Parasitology**, v. 43, n. 3, p. 488-490, 1957.

ELDIN, H. M. E.; BADAWY, A. F. *In vitro* anti-*Trichomonas vaginalis* activity of *Pistacia lentiscus* mastic and *Ocimum basilicum* essential oil. **Journal of Parasitogoly Diseases**, v. 39, n. 3, p. 465-473, 2015.

FENEQUE, J. Nanotechnology: a new challenge contributing to the creation of changes in veterinary for veterinary medicine. **The Pet Tribune**, v. 6, n. 5, p. 16, 2000.

FLORES, F. C. *et al*. Nanostructured systems containing an essential oil: protection against volatilization. **Química Nova**, v. 34, n. 6, p. 968-972, 2011.

FORRESTER, D. J.; FOSTER, G. W. Trichomonosis. *In*: ATKINSON, C. T.; THOMPSON, N. J.; HUNTER, D. B. (Ed.). **Parasitic diseases of wild birds**. Ames: Wiley-Blackwell, 2008.

FRANSSEN, F. F. J.; LUMEIJ, J. T. *In vitro* nitroimidazole resistance of *Trichomonas gallinae* and successful therapy with an increased dosage of ronidazole in racing pigeons (*Columba livia* domestica). **Journal of Veterinary Pharmacology and Therapeutics**, v. 15, n. 4, p. 409-415, 1992.

FRONZA, T. *et al*. Nanoemulsões como sistemas de liberação para fármacos oftálmicos. **Acta Farmacéutica Bonaerense**, v. 23, n. 4, p. 558-556, 2004.

GERHOLD, R. *et al*. Molecular characterization of the *Trichomonas gallinae* morphologic complex in the United States. **Journal of Parasitology**, v. 94, n. 6, p. 1335-41, 2008.

GODOI, S. N. *et al*. Evaluation of stability and *in vitro* security of nanoemulsions containing *Eucalyptus globulus* oil. **BioMed Research International**, v. 17, p. 1-10, 2017.

GONZA´LEZ-COLOMA, A. *et al*. Antileishmanial, antitrypanosomal and cytotoxic screening of ethnopharmacologically selected Peruvian plants. **Parasitology Research**, v. 110, n. 4, p. 1381-1392, 2011.

GRADONI, L.; ASCENZI, P. Nitric oxide and anti-protozoan chemotherapy. **Parassitologia**, v. 46, n. 1-2, p. 101-3, 2004.

GÜNDEL, S. S. *et al*. Basil oil-nanoemulsions: development, cytotoxicity and evaluation of antioxidant and antimicrobial potential. **Journal of Drug Delivery Science and Technology**, v. 46, p. 378-383, 2018.

HAMMER, K. A. *et al*. Frequencies of resistance to *Melaleuca alternifolia* (tea tree) oil and rifampicin in *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*. **International Journal of Antimicrobial Agents**, v. 32, n. 2, p. 170-3, 2008.

HARLIN, R.; WADE, L. Bacterial and parasitic diseases of Columbiformes. **Veterinary Clinics of North America:** Exotic Animal Practice, v. 12, n. 3, p. 453-73, 2009.

HUSSAIN, A. I. *et al*. Chemical composition, antioxidant and antimicrobial activities of essential oil of spearmint (Mentha spicata L.) from Pakistan. **Journal of Essential Oil Research**, v. 22, n. 1, p. 78-84, 2010.

IBRAHIM, A. N. Comparison of *in vitro* activity of metronidazole and garlic-based product (Tomex®) on *Trichomonas vaginalis*. **Parasitology Research**, v. 112, n. 5, p. 2063-7, 2013.

JITVIRIYANON, S. *et al*. *In vitro* study of anti-coccidial activity of essential oils from indigenous plants against *Eimeria tenella.* **Veterinary Parasitology***,* v. 228, p. 96-102, 2016.

KANOJIYA, D. *et al*. *In vitro* and in vivo efficacy of extracts of leaves of Eucalyptus globulus on ovine gastrointestinal nematodes. **Parasitology Research**, v. 114, n. 1, p. 141-148, 2014.

KOCAN, R. M. Various grains and liquid as potential vehicles of transmission for *Trichomonas gallinae*. **Bulletin of the Wildlife Disease Association**, v. 5, n. 3, p. 148-9, 1969.

KRONE, O. *et al*. Prevalence of *Trichomonas gallinae* in northern goshawks from the Berlin area of northeastern Germany. **Journal of Wildlife Diseases,** v. 41, n. 2 p. 304-9, 2005.

LEONG, T. S. H. *et al*. Minimising oil droplet size using ultrasonic emulsification. **Ultrasound Sonochemistry**, v. 16, n. 6, p. 721-727, 2009.

LUMEIJ, J. T.; ZWIJNENBERG, R. J. G. Failure of nitro-imidazole drugs to control trichomoniasis in the racing pigeon (*Columba livia* domestica). **Avian Pathology**, v. 19, p. 165-166, 1990.

MARTINEZ-VELAZQUEZ, G. A. *et al*. Acaricidal effect and chemical composition of essential oils extracted from *Cuminum cyminum*, *Pimenta dioica* and *Ocimum basilicum* against the cattle tick *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae). **Parasitology Research**, v. 108, n. 2, p. 481-487, 2011.

MARUNO, M.; ROCHA-FILHO, P. A. O/W nanoemulsion after 15 years of preparation: A suitable vehicle for pharmaceutical and cosmetic applications. **Journal of Dispersion Science and Technology**, v. 31, p. 17-22, 2010.

MATOS, F. J. de *et al*. Craveiro. **Constituintes Químicos Ativos e Propriedades Biológicas de Plantas Medicinais Brasileiras**. 2. ed. Fortaleza: EDIÇÕES UFC, 2004.

MCCLEMENTS, D. J. Nanoemulsions versus microemulsions: terminology, differences, and similarities. **Soft Matter**, v. 8, p. 1719-1729, 2012.

MEHLHORN, H. *et al*. Entomological survey on vectors of Bluetongue virus in North Rhine-Westphalia (Germany during 2007–2008). **Parasitology Research**, v. 105, n. 2, p. 321-9, 2009.

MUNOZ, E. *et al*. *In vivo* and *in vitro* sensitivity of *Trichomonas gallinae* to some nitroimidazole drugs. **Veterinary Parasitology**, v. 78, n. 4, p. 239-46, 1998.

NAIR, J. *et al*. A nutritional evaluation of common barley varieties grown for silage by beef and dairy producers in western Canada. **Canadian Journal of Animal Science**, v. 96, n. 4, p. 598-608, 2016.

NIKPAY, A.; SOLTANI, M. *In vitro* anti-parasitic activities of *Pulicaria dysenterica* and *Lycopus europaeus* methanolic extracts against *Trichomonas gallinae*. **Journal of Herbmed Pharmacology**, v. 7, n. 2, p. 112-118, 2018.

PURPLE, K.; GERHOLD, R. Persistence of Two Isolates of *Trichomonas gallinae* in Simulated Bird Baths With and Without Organic Material. **Avian Diseases**, v. 59, n. 4, p. 472-474, 2015.

ROBINSON, R. A. *et al*. The finch epidemic strain of *Trichomonas gallinae* is predominant in British non-passerines. Emerging infectious disease leads to rapid population declines of common British birds. **Plos One**, v. 5, n. 8, p. 1234-1245, 2010.

ROUFFAER, L. *et al*. Racing pigeons: a reserv3oir for nitro-imidazole-resistant *Trichomonas gallinae*. **The Journal of Parasitology**, v. 100, n. 3, p. 360-3, 2014.

SALAMANCA-BUENTELLO, F. *et al*. Nanotechnology and the Developing World. **PLoS Med**, v. 2, n. 5, p. 97, 2005.

SCHMALTZ, C. *et al*. Nanocápsulas como uma tendência promisora na área cosmética: a imensa potencialidade deste pequeno grande recurso. **Infarma**, v. 16, n. 13-14, p.80-85, 2005.

SEDDIEK, A. *et al*. The antitrichomonal efficacy of garlic and metronidazole against *Trichomonas gallinae* infecting domestic pigeons. **Parasitology Research**, v. 113, n. 4, p. 1319-1329, 2014.

SENA-LOPES, Â. *et al*. Antiparasitic activity of 1,3-dioxolanes containing tellurium in *Trichomonas vaginalis*. **Biomedicine ; Pharmacotherapy**, v. 89, p. 284-287, 2017.

SILVA, G. A. Introduction to nanotechnology and its applications to medicine. **Surgical Neurology**, v. 61, n. 3, p. 216-220, 2004.

SILVA, J. *et al*. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. **Journal of Ethnopharmacology**, v. 89, p. 277-283, 2003.

SOBEL, J.D.; KAYE, D. Urinary tract infections. In: Mandell Gl, Bennett JC, Dolin R., editors. Mandell, Douglas, and Bennett's: **Principles and Practice of Infectious Disease**. 7. Elsevier; PA, USA: 2010. p. 957–985.

SOLANS, C. *et al*. Nano-emulsions. **Current Opinion in Colloid ; Interface Science**, v. 10, n. 3-4, p. 102-110, 2005.

SOLÓRZANO-SANTOS, F.; MIRANDA-NOVALES, M. G. Essential oils from aromatic herbs as antimicrobial agents. **Current Opinion in Biotechnology**, v. 23, n. 2, p. 136-41, 2012.

STABLER, R. M. *Trichomonas gallinae*. A review. **Experimental Parasitology**, v. 3, n. 4, p. 368-402, 1954.

STOCKDALE, J. E. *et al*. The protozoan parasite *Trichomonas gallinae* causes adult and nestling mortality in a declining population of European Turtle Doves, Streptopelia turtur. **Parasitology**, v.142, n. 3, p. 490-498, 2015.

TABARI, M. A. *et al*. Antitrichomonal activity of *Peganum harmala* alkaloid extract against trichomoniasis in Pigeon (*Columba livia* domestica), **British Poultry Science**, v. 58, n. 3, p. 236-241, 2017.

TRADOS, T. *et al*. Formation and stability of nano-emulsions. **Advances in colloid and Interface Science**, v. 108-109, p. 303-318, 2004.

VITTI, A. M. S.; BRITO, J. O. **Óleo essencial de eucalipto**. São Paulo: ESALQ/USP, 2003. 26p.

WEN-CHIEN, L. *et al*. Preparation, characterization, and antimicrobial activity of nanoemulsions incorparating citral essential oil. **Journal of Food and Drug Analysis**, v. 26, n. 1, p. 82-89, 2018.

XAVIER, M. N. *et al*. Composição química do óleo essencial de *Cardiopetalum calophyllum* Schltdl. (Annonaceae) e suas atividades antioxidante, antibacteriana e antifúngica. **Revista Virtual de Química**, v. 8, n. 5, p. 1433, 2016.

YOUSSEFI, M. R. *et al*. *In vitro* and *in vivo* activity of *Artemisia sieberi* against *Trichomonas gallinae*. **Iran Journal of Veterinary Research**, v. 18, n. 1, p. 25-29, 2017.