DOI: 10.5216/cab.v14i4.22080

Origanum vulgare (Lamiaceae) OVICIDAL POTENTIAL ON GASTROINTESTINAL NEMATODES OF CATTLE

LUCIANA LAITANO DIAS DE CASTRO[1], ISABEL MARTINS MADRID[1], CÍNTIA LIDIANE GUIDOTTI AGUIAR[1], LEONARDO MORTAGUA DE CASTRO[1], MARLETE BRUM CLEFF[2], MARIA ELISABETH AIRES BERNE[1], FÁBIO PEREIRA LEIVAS LEITE[3]

[1]Departamento de Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brasil - luciana_ldc@hotmail.com
[2]Departamento de Clínicas Veterinária, Faculdade de Veterinária, UFPEL, Pelotas, RS, Brasil
[3]Núcleo de Biotecnologia, Centro de Desenvolvimento Tecnológico, UFPEL, Pelotas, RS, Brasil

ABSTRACT

Due to anthelmintic resistance in nematodes, several research studies have been developed seeking control alternatives to these parasites. This study evaluated the *in vitro* action of *Origanum vulgare* on gastrointestinal nematode eggs of cattle. In order to evaluate the ability to inhibit egg hatch, different dried leaves extracts of this plant were tested, such as dye, hydroalcoholic and aqueous extracts at concentrations varying from 0.62 to 80 mg/mL. Each assay was accompanied by control containing levamisole hydrochloride (0.2 mg/mL), distilled water and 70º GL grain alcohol at the same concentration of the extracts. Test results showed that the different *O. vulgare* extracts inhibited egg hatch of cattle gastrointestinal nematodes at a percentage that varied from 8.8 to 100%; dye and hydroalcoholic extract were the most promising inhibitors. In view of this ovicidal property, *O. vulgare* may be an important source of viable antiparasitic compounds for nematodiosis control in ruminants.

KEYWORDS: *Haemonchus* spp.; in vitro; oregano; phytotherapic.

POTENCIAL OVICIDA DE O*riganum vulgare* (Lamiaceae) EM NEMATÓDEOS GASTRINTESTINAIS DE BOVINOS

RESUMO

Em virtude da resistência dos nematódeos gastrintestinais aos antihelmínticos, diversas pesquisas têm sido desenvolvidas buscando-se alternativas de controle para essas parasitoses. Nesse contexto, o estudo avaliou a ação *in vitro* de *Origanum vulgare* sobre ovos de nematódeos gastrintestinais de bovinos. Para avaliar a capacidade de inibição da eclodibilidade dos ovos, diferentes formas de extratos das folhas secas desta planta foram testadas como tintura, extrato hidroalcoólico e extrato aquoso nas concentrações de 0,62 a 80 mg/mL. Cada ensaio foi acompanhado de controle contendo cloridrato de levamisol (0,2 mg/mL), água destilada e álcool de cereais 70° GL nas mesmas concentrações dos produtos. Os resultados dos testes demonstraram que os diferentes extratos de *O. vulgare* inibiram a eclodibilidade dos ovos de nematódeos gastrintestinais de bovinos com percentual de inibição variando de 8,8 a 100%, sendo a tintura e o extrato hidroalcoólico as formas mais promissoras. Frente a esta propriedade ovicida, o *O. vulgare* pode representar uma importante fonte de compostos antiparasitários viáveis para o controle das nematodioses em ruminantes.

PALAVRAS-CHAVE: Fitoterápico; *Haemonchus* spp.; *in vitro*; orégano

INTRODUCTION

Gastrointestinal helminthic diseases in ruminants are among the main problems that affect livestock in this raising system, greatly limiting the economic exploitation of these animals (COUMENDOUROS et al., 2003). The administration of anthelmintic drugs to these animals is the main control measure to prevent the economic losses caused by the parasites (MILLER & HOROHOV, 2006); however, the wide use of these drugs has contributed to the emergence of resistant nematodes, which is a worldwide problem (CHAGAS et al., 2011). In addition to this, there has been a great demand, both within the country and abroad, for animal products which are free from chemical residues; among the natural alternatives for ruminant gastrointestinal nematode control are medicinal plants, whose studies have shown promising results (ALMEIDA et al., 2007; MACEDO et al., 2009; KAMARAJ et al., 2011).

Until the emergence of synthetic products, most available drugs in the world originated from studies developed based on the popular culture, which makes the rich Brazilian biodiversity a vast field of research (BRASIL, 2011). The Lamiaceae family, in turn, includes the third largest number of species listed as having antiparasitic activity (FURTADO, 2006). Research developed with plants of this family, especially with *Origanum vulgare* essential oil, has demonstrated antiprotozoal activity against *Trypanossoma cruzi* (SANTORO et al., 2007) and *Eimeria tenella* (GIANNENAS et al., 2003). Several studies on helminths have also been done to evaluate *O. vulgare* essential oil action against phytopathogens (OKA et al., 2000; BARBOSA et al., 2010) and human intestinal parasites (FORCE et al., 2000), although there are no studies testing the effect of the *O. vulgare* on gastrointestinal nematodes of cattle. Therefore, the main goal of this study was to evaluate the *in vitro* action of different dried leaves extracts of *O. vulgare* on gastrointestinal nematode eggs of cattle.

MATERIALS AND METHODS

O. vulgare dry leaves with quality and origin certification were obtained from a commercial distributor (Luar Sul®). Different forms of plant extracts, such as dye, hydroalcoholic and aqueous extracts, were prepared and tested.

The dye was prepared at a 0.1 g plant concentration per microliter of 70° GL grain alcohol (GA) as described by Schiedeck et al. (2008), and stir-macerated daily for a seven-day period. After this period, the solution was filtered through a filter paper (n. 1 Whatman) to eliminate solid residue; the initial volume was restored with 70° GL grain alcohol, and stored in an ambar vial for later use. The solvent was extracted at 55 ºC under 600 mm/Hg negative pressure using rotary evaporation (Q344M Quimis) to obtain the hydroalcoholic extract (HAE). Later, the initial volume was restored with sterile distilled water, and HAE was used soon afterwards.

To produce aqueous extract (AqE) a 10% solution was prepared from the infusion of the plant in water at 90 ºC, and kept in closed flasks for 10 minutes. Following, the samples were filtered through filter paper (n. 1 Whatman) to eliminate solid residues; the initial volume was restored with sterile distilled water and the solution was used immediately.

Eggs were obtained by collecting feces directly from the rectal ampulla of 120-day old Holstein cattle, which presented a gastrointestinal nematode mixed infection. The animals were maintained without anthelminthic treatment for a 60-day period prior to collection; the count of eggs per gram of feces (EPG) was obtained by the GORDON & WHITLOCK (1939) technique for over 2000 eggs. An adapted version of the HUBERT & KERBOEUF (1992) technique was used for the recovery of eggs, when feces were macerated, diluted in distilled water and passed through four sieves in decreasing mesh opening order (1 mm, 105 µm, 55 µm, 25 µm). The eggs were recovered from the 25 µm sieve, diluted in distilled water and quantified from a 50 µL suspension aliquot three times.

The genera of the nematode larvae present in the feces were determined by the ROBERTS & O'SULLIVAN (1950) technique.

The egg hatch test was performed in 24-well microplates with six replicates, according to the technique proposed by COLES et al. (1992). Approximately 150 eggs were placed in each well and the extract was tested in eight successive log2 concentrations of 80 to 0.62 mg/mL. As control, 0.2 mg/mL levamisole hydrochloride, sterile distilled water, and 70° GL grain alcohol (dye solvent) at the same concentrations as those of the extracts, were used.

The microplates were incubated in a BOD incubator at 28ºC at a relative humidity of 80% for 24 h for quantification of eggs and first stage larvae in an inverted microscope (Zeiss, Germany). Results were expressed as the sextuplicate mean percentage of hatchability inhibition, and the

efficacy of each treatment in the egg hatch test was determined according to the equation described by CAMURÇA-VASCONCELOS et al. (2007):

$$Hatchability\ inhinbition\ percentage = \frac{number\ of\ larvae}{(number\ of\ larvae + numebr\ of\ eggs)} 100$$

The results were analyzed by ANOVA and means were compared by the Tukey test (P≤0.05) by using the Statistix 9.0 software.

RESULTS AND DISCUSSION

The different *O. vulgare* extracts inhibited egg hatch gastrointestinal nematodes of cattle, with an inhibition percentage ranging from 8.8 to 100% (Table 1). In addition, the sextuplicate results were homogeneous in all tests performed, showing a low standard deviation. The distilled water and anthelminthic controls showed an average percentage inhibition of 9.8% and 100%, respectively. The fecal culture result revealed the occurrence of the genera *Haemonchus* (83%), *Trichostrongylus* (16%) and *Oesophagostomum* (1%).

Table 1. Mean inhibition percentage ± standard deviation of gastrointestinal nematodes eggs hatch of cattle in different treatments and concentrations of *Origanum vulgare* extracts

Concentrations (mg/mL)	Extracts			
	GA	Dye	HAE	AqE
80	100 ^{Aa} ± 0.0	100 ^{Aa} ± 0.0	96.7 ^{Ba} ± 1.51	49.8 ^{Ca} ± 2.24
40	100 ^{Aa} ± 0.0	100 ^{Aa} ± 0.0	80 ^{Bb} ± 1.44	27.7 ^{Cb} ± 4.65
20	95.8 ^{Bb} ± 0.67	100 ^{Aa} ±0.0	58.7 ^{Cc} ± 0.98	18.8 ^{Dc} ± 0.76
10	27.1 ^{Cc} ± 1.74	95.6 ^{Ab} ± 1.11	41.6 ^{Bd} ± 3.25	15.7 ^{Dc} ± 0.72
5	14.2 ^{Bd} ± 2.19	40.6 ^{Ac} ± 0.92	11.6 ^{BCe} ± 0.85	9.6 ^{Cd} ± 1.71
2.5	14.2 ^{Bd} ± 0.42	41.4 ^{Ac} ± 0.93	11 ^{Ce} ± 0.86	9.5 ^{Cd} ± 2.57
1.25	15.6 ^{Bd} ± 1.36	33.4 ^{Ad} ± 3.36	10.7 ^{Ce} ± 2.72	9.8 ^{Cd} ± 0.41
0.62	11.5 ^{Be} ± 0.57	30.1 ^{Ad} ± 2.96	11.3 ^{Be} ± 1.64	8.8 ^{Bd} ± 3.25

GA – grain alcohol 70° GL; HAE - hydroalcoholic extract; AqE – aqueous extract.
Different capital letters, in the line, and different small letters, in the column, differ significantly from each other (p≤0.05).

The hatchability inhibition values for the dye ranged from 100 to 30.1%; this extract showed good activity, showing similar results to anthelminthic control at the 80, 40 and 20 mg/mL concentrations (p>0.05). In the consecutive concentrations there was a statistically significant difference in inhibition percentages among the concentrations 10, 5 and 1.25 mg/mL, this effect was directly proportional to the concentration (p<0.0001). From the concentration of 10 mg/mL on, one can observe a significant difference between the GA and dye inhibition rates (p<0.05). The active compounds present in the dye explain this increase.

Grain alcohol (GA) also obtained results ranging from 100 to 11.5%, showing a significantly lower action than the dye (p<0.0001) for the 20 mg/mL concentration, when the dye obtained a 100% inhibition rate and GA, 95.8%. At the 10 mg/mL concentration, dye action was 3.5 times higher than GA; for this concentration, the inhibition difference was on average 2.6 higher for the dye as compared to GA.

The average inhibition hatchability percentage of the *O. vulgare* hydroalcoholic extract at the 80 mg/mL concentration was 96.7%, only 3.3% lower than that of the anthelmintic control. A gradual decrease of ovicidal activity was found at consecutive concentrations, with statistical differences between concentrations until 5 mg/mL. Below this concentration, the average inhibition percentage remained lower than 11.6%, not showing statistical differences from consecutive concentrations and the distilled water control (p>0.05).

The results of the *O. vulgare* aqueous extract demonstrated egg hatch inhibition, although the average inhibition percentage was low, ranging from 49.8 to 8.8% at the concentrations tested. At the 80 mg/mL concentration, the aqueous extract revealed a 49.8% efficacy, showing a lower activity than that of

the anthelmintic control ($p<0.0001$) and higher than that of the distilled water control ($p<0.0001$). When the 80 and 40 mg/mL concentrations were compared, a significant activity reduction to 27.7% was observed ($p<0.0001$). From the 5 mg/mL concentration, this effect remained lower than 10%, showing no statistically significant difference from the distilled water control ($p>0.05$).

In vitro tests have been widely used in the screening of medicinal plants, offering advantages such as ease of application, low cost, speed, in addition to preventing the indiscriminate use of experimental animals (CAMURÇA-VASCONCELOS et al., 2005). The results obtained in this study showed that *O. vulgare* presents a differentiated ovicidal effect on cattle nematodes. These differences are related to different factors, especially active principles extracted by using different techniques and solvents, as well as a variation in solubility of the active constituents in solvent systems (ELOFF, 1998).

A range of active compounds, including terpenoids, flavanoids, tannins and carvacol, considered as a potential source of natural bioactive elements, are present in the aromatic plant *O. vulgare* (CLEFF et al., 2010). Several studies report that the presence of flavonoids and tannins is responsible for the its anthelmintic activity (ATHNASIADOU et al., 2001; KERBOEUF et al., 2008). The presence of these various compounds in the leaf of *O. vulgare* could then be responsible for the anthelmintic activities of the extracts tested in this study.

Even though no studies have showed the action of *O. vulgare* extracts on nematode of cattle, some research studies evaluating the action of plants from the same family on other parasites were found. GARDIANO et al. (2011), upon evaluating the anthelmintic potential of *O. vulgare* aqueous extract at a 100 mg/mL concentration on the phytonematode *Rotylenchulus reniformis*, observed an egg count reduction of only 28%. This result was lower than that of this study, which obtained a 49.8% egg hatch inhibition at the 80 mg/mL concentration.

The dye and hydroalcoholic extract of *O. vulgare* showed similar activity to other plants of the same family. An example of this is the extract of the plant *Anisomeles malabarica* R. Br., which, at a 25 mg/mL concentration, inhibited the hatch of *H. contortus* eggs at 100, 93.8 and 83.2% when extracted with ethyl acetate, methanol and acetone solvents, respectively (KAMARAJ et al., 2011), while *Mentha piperita* extract at a 0.078 mg/mL concentration inhibited approximately 90% of *H. contortus* egg hatch (CARVALHO et al., 2012). *Mentha villosa* hydrolate, in turn, at concentrations ranging from 20 to 100%, inhibited egg hatch of gastrointestinal nematodes of cattle between 67 and 100%, which was a dose-dependent effect (NASCIMENTO et al., 2009). EGUALE et al. (2011) found 100% hatchability inhibition of *Haemonchus contortus* eggs when either the aqueous or hydroalcoholic extract of the plant *Leucas martinicensis* (Lamiaceae) at a 1 mg/mL concentration was used.

According to the Brazilian Pharmacopoeia Herbal Formulary, several dyes formulated with 70% alcohol, which have anti-inflammatory, antiflatulent, antispasmodic, diuretic, antiseptic, expectorant, scabicide, and pediculicide action, are recommended for internal and external use in humans (BRASIL, 2011). In this sense, a lower *O. vulgare* dye concentration (10 mg/mL) with ovicidal action may be used in helminthiases because it contains 7% alcohol in its composition, but its action *in vivo* still needs to be tested.

The dye can be considered the most viable option for popular use insofar as this formulation involves a simple, easy to handle technique, which does not require specific equipment, and the material can be stored for up to two years (SCHIEDECK et al., 2008). Besides, medicinal plant dyes have been widely used for the treatment of several diseases in popular medicine, as well as for phytopathogen control (SOUZA et al., 2004; SCHIEDECK et al., 2008).

CONCLUSIONS

The different *O. vulgare* extracts used in this study showed anthelmintic activity on eggs of gastrointestinal nematodes of cattle; among them, dye and hydroalcoholic extract at 10 mg/mL and 80 mg/mL, respectively, showed the most promising results. However, further studies are necessary to evaluate their toxicity and active components, in addition to their *in vivo* anthelmintic potential.

REFERENCES

ALMEIDA, M.A.O.; DOMINGUES, L.F.; ALMEIDA, G.N.; SIMAS, M.M.S.; BOTURA, M.B.; CRUZ, A.C.F.G.; SILVA, A.V.A.F.; MENEZES, T.P.; BATATINHA, M.J.M. Efeitos dos extratos aquosos de folhas de *Mentha piperita* L. e de *Chenopodium ambrosioides* L. sobre cultivos de larvas infectantes de nematóides gastrintestinais de caprinos. **Revista Brasileira de Parasitologia Veterinária**, v.16, n.1, p.57-59, 2007.

ATHNASIADOU, S.; KYRIAZAKIS, I.; JACKSON, F.; COOP, R.L. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of

sheep: *in vitro* and *in vivo* studies. **Veterinary Parasitology,** v.99, p.205–219, 2001.

BARBOSA, P.; LIMA, A.S.; VIEIRA, P.; DIAS, L.S.; TINOCO, M.T.; BARROSO, J.G.; PEDRO, L.G; FIGUEIREDO, A.C.; MOTA, M. Nematicidal activity of essential oils and volatiles derived from Portuguese aromatic flora against the pinewood nematode, *Bursaphelenchus xylophilus.* **Journal of Nematology**, v.42, n.1, p.8-16, 2010.

BRASIL - Agência Nacional de Vigilância Sanitária, **Formulário de Fitoterápicos da Farmacopéia Brasileira**. Brasília: Anvisa, 2011. Disponível em http://www.anvisa.gov.br/hotsite/farmacopeiabrasileira/conteudo/Formulario_de_Fitoterapicos_da_Farmacopeia_Brasileira.pdf, acesso em novembro de 2013.

CAMURÇA-VASCONCELOS, A.L.F.; MORAIS, S.M.; SANTOS, L.F.L.; ROCHA, M.F.G.; BEVILAQUA, C.M.L. Validação de plantas medicinais com atividade anti-helmíntica. **Revista Brasileira de Plantas Medicinais**, v.7, n.3, p.97-106, 2005.

CAMURÇA-VASCONCELOS, A.L.F.; BEVILAQUA, C.M.L.; MORAIS, S.M.; MACIEL, M.V.; COSTA, C.T.C.; MACEDO, I.T.F.; OLIVEIRA, L.M.B.; BRAGA, R.R.; SILVA, R.A.; VIEIRA L.S. Anthelmintic activity of *Croton zehntneri* and *Lippia sidoides* essential oils. **Veterinary Parasitology**, v.148, n.3-4, p.288-294, 2007.

CARVALHO, C.O.; CHAGAS, A.C.S.; COTINGUIBA, F.; FURLAN, M.; BRITO, L.G.; CHAVES, F.C.M.; STEPHAN, M.P.; BIZZO, H.R.; AMARANTE, A.F.T. The anthelmintic effect of plant extracts on *Haemonchus contortus* and *Strongyloides venezuelensis*. **Veterinary Parasitology**, v.183, p.260-268, 2012.

CHAGAS, A.C.S.; NICIURA, S.C.M.; MOLENTO, M.B. **Manual Prático**: metodologias de diagnóstico da resistência e de detecção de substâncias ativas em parasitas de ruminantes. Brasília: Embrapa Informação Tecnológica, 2011. Disponível em: http://livraria.sct.embrapa.br/liv_resumos/pdf/00061850.pdf

CLEFF, M.B.; MEINERZ, A.R.M.; XAVIER, M.; SCHUCH, L.F.; MEIRELES, M.C.A.; RODRIGUES, M.R.A.; MELLO, J.R.B. *In vitro* activity of *Origanum vulgare* essential oil against *Candida* species. **Brazilian Journal of Microbiology**, v.41, n.1, p.116-123, 2010.

COLES, G.C.; BAUER, C.; BORGSTEEDE, F.H.M.; GEERTS, S.; KLEI, T.R.; TAYLOR, M.A.; WALLER, P.J. World Association for the advancement of Veterinary Parasitology (W.A.A.V.P.) methods for detection of anthelmintic resistance in nematodes of veterinary importance. **Veterinary Parasitology**, p.44, n.1-2, p.35-44, 1992.

COUMENDOUROS, K.; TANCREDI, I.P.; SCOTT, F.B.; MARTINS, I.V.F.; SANT'ANNA, F.B.; GRISI, L. Eficácia anti-helmíntica da eprinimectina no controle de nematoides gastrintestinais de bovinos. **Revista Brasileira de Parasitologia Veterinária**, v.12, n.3, p.121-124, 2003.

EGUALE, T.; TADESSE, D.; GIDAY, M. *In vitro* anthelmintic activity of crude extracts of five medicinal plants against egg-hatching and larval development of *Haemonchus contortus*. **Journal of Ethnopharmacology**, v.137, n.1, p.108-113, 2011.

ELOFF, J.N. Which extractant should be used for screening and isolation of antimicrobial components from plants? **Journal Ethnopharmacology**, v.60, n.1, p.1-8, 1998.

FORCE, M.; SPARKS, W.S.; RONZIO, R.A. Inhibition of Enteric Parasites by Emulsified Oil of Oregano *in vivo*. **Phytotherapy Research**, v.14, n.3, p.213 -214, 2000.

FURTADO, S.K. **Alternativas fitoterápicas para o controle da verminose ovina no estado do Paraná**: testes *in vitro e in vivo*. Tese, Universidade Federal do Paraná, Brasil, 2006. Disponível em: http://www.oikos.ufpr.br/publicacoes/teses/01_Tese%20Silvana%20Furtado.pdf

GARDIANO, C.G.; MURAMOTTO, S.P.; KRZYZANOWISKI, A.A.; ALMEIDA, W.P.; SAAB, O.J.G.A. Efeito de extratos aquosos de espécies vegetais sobre a multiplicação de *Rotylenchulus reniformis* Linford & Oliveira. **Arquivos do Instituto Biológico**, v.78, n.4, p.553-556, 2011.

GIANNENAS, I.; FLOROU-PANERI, P.; PAPAZAHARIADOU, M.; CHRISTAKI, E.; BOTSOGLOU, N.A.; SPAIS, A.B. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. **Archives fur Tierernahrung**, p.57, n.2, p.99-106, 2003.

GORDON, H.M.; WHITLOCK, H.V. A new technique for counting nematode eggs in sheep faeces. **Journal of the Council for Scientific and Industrial Research**, v.12, n.1, p.50-52, 1939.

HUBERT, J.; KERBOEUF, D.A. Microlarval development assay for the detection of anthelmintic resistance in sheep nematodes. **Veterinary Research**, v.130, n.20, p.442-446, 1992.

KAMARAJ, C.; RAHUMAN, A.A.; ELANGO, G.; BAGAVAN, A.; ZAHIR A.A. Anthelmintic activity of botanical extracts against sheep gastrointestinal nematodes, *Haemonchus contortus*. **Parasitology Research**, v.109, n.1, p.37-45, 2011.

KERBOEUF, D.; RIOU, M.; GUÉGNARD, F. Flavonoids and related compounds in parasitic disease control. **Mini-Reviews Medicinal Chemmistry** v.8, p.116–128, 2008.

MACEDO, I.T.F.; BEVILAQUA, C.M.L.; OLIVEIRA, L.M.B.; CAMURÇA-VASCONCELOS, A.L.F.; VIEIRA, L.S.; OLIVEIRA, F.R.; QUEIROZ-JUNIOR, E.M.; PORTELA, B.G.; BARROS, R.S.; CHAGAS, A.C.S. Atividade ovicida e larvicida *in vitro* do óleo essencial de *Eucalyptus globulus* sobre *Haemonchus contortus*. **Revista Brasileira de Parasitologia Veterinária**, v.18,

n.3, p.62-66, 2009.

MILLER, J.E.; HOROHOV, D.W. Immunological aspects of nematode parasite control in sheep. **Journal of Animal Science**, v.84, (suplem E), p.124-132, 2006.

NASCIMENTO, E.M.; FURLONG, J.; PIMENTA, D.S.; PRATA, M.C.A. Efeito anti-helmíntico do hidrolato de *Mentha villosa* Hunds. (Lamiaceae) em nematoides gastrintestinais de bovinos. **Ciência Rural**, v.39, n.3, p.817-824, 2009.

OKA, Y.; KOLTAI, H.; BAR-EYAL, M.; MOR, M.; SHARON, E.; CHET, I.; SPIEGEL, Y. New strategies for the control of plant-parasitic nematodes. **Pest Management Science**, v.56, p.983-988, 2000.

ROBERTS, F.H.S.; O'SULLIVAN, S.P. Methods for egg counts and larvae cultures for strongyles infesting the gastrointestinal tract of cattle. **Australian Journal of Agricultural Research**, v.1, p.99-102, 1950.

SANTORO, G.F.; CARDOSO, M.G.; GUIMARÃES, L.G.; FREIRE, J.M.; SOARES, M.J. Anti-proliferative effect of the essential oil of *Cymbopogon citratus* (DC) Stapf (lemongrass) on intracellular amastigotes, bloodstream trypomastigotes and culture epimastigotes of *Trypanosoma cruzi* (Protozoa: Kinetoplastida). **Parasitology**, v.134, p.1649-1656, 2007.

SOUZA, G.C.; HAAS, A.P.S.; VON POSER, G.L.; ELISABETSKY, R. Farmácias caseiras comunitárias do município de Maquiné (RS): uma avaliação etnofarmacológico. **Revista Brasileira de Plantas Medicinais**, v.6, n.2, p.83-91, 2004.

SCHIEDECK, G.; BEVILAQUA, G.A.P.; NACHTIGAL, G.F.; BAUER, M.V. Método de preparo de tintura de plantas bioativas para fins agrícolas. **EMBRAPA – Comunicado Técnico**, v.190, p.1-4, 2008. Disponível em: http://www.infoteca.cnptia.embrapa.br/bitstream/doc/746473/1/comunicado190.pdf

Protocolado em:15 jan. 2013. Aceito em: 30 jul. 2013.