

NOTAS PRÉVIAS

Efficacy of essential oil of *Lippia origanoides* in the disinfection of teats of dairy cows and milking utensils

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ABSTRACT: The antimicrobial efficiency of essential oil of the *Lippia origanoides* was held in the presence of organic matter in teats of dairy cows and milking utensils. *Staphylococcus aureus* ATCC 25923, *Salmonella choleraesuis* ATCC 10708 and *Escherichia coli* ATCC 8739 were in contact with the oil by 5 min in suspension test with sterile powdered milk used as organic matter. Spray containing solution 120 µL/mL of the oil was applied to teats and milking utensils and the results compared with the control group. In suspension test the essential oil showed activity in the presence of organic matter, being able to eliminate bacteria after 5 min of contact. The oil solution was able to significantly reduce the count of coagulase positive *Staphylococcus* spp and fecal coliforms in the teats ($p < 0,0001$), and aerobic mesophilic in milking utensils ($p < 0,0001$) with 5 min of contact. These results indicate that the essential oil of *L. origanoides* can be an alternative to chemicals that are commonly used in the management of milking dairy cows.

Keywords: milk, sanitation, biocides, hygiene, disinfectants.

RESUMO: Eficácia do óleo essencial de *Lippia origanoides* na desinfecção de tetos de vacas leiteiras e utensílios de ordenha. A eficiência antimicrobiana de óleo de alecrim pimenta (*Lippia origanoides*) foi avaliada na presença de matéria orgânica em tetos de vacas leiteiras e utensílios de ordenha. Cepas de *Staphylococcus aureus* ATCC 25923, *Salmonella choleraesuis* ATCC 10708 e *Escherichia coli* ATCC 8739 estiveram em contato com o óleo no tempo de 5 min para o teste de suspensão, juntamente com o leite desnatado estéril utilizado como matéria orgânica. Um spray contendo solução de 120 µL/mL do óleo foi aplicado em tetos, teteiras e latões utilizados no armazenamento de leite e os resultados obtidos foram comparados com o grupo controle. No teste de suspensão, o óleo essencial de *Lippia origanoides* apresentou atividade na presença de matéria orgânica, sendo capaz de eliminar as bactérias após 5 min de contato. A solução de óleo de alecrim pimenta foi capaz de reduzir significativamente a contagem de *Staphylococcus* spp coagulase positiva e de coliformes termotolerantes nos tetos ($P < 0,0001$) e de mesófilos aeróbios nas teteiras e nos latões ($P < 0,0001$) utilizados na ordenha com 5 min de contato, tornando-os uma alternativa aos compostos químicos que são comumente utilizados.

Palavras-chave: leite, sanitização, biocidas, higiene, desinfetantes.

INTRODUCTION

Biocides are compounds that are frequently used in food-processing environments to reduce or eliminate pathogenic and spoilage microorganisms, among other functions. This practice has generated concerns about the potential of microorganisms to develop resistance to these compounds as an

adaptation mechanism to environmental conditions (Alonso-Calleja, *et al.*, 2015). This tolerance may facilitate the prevalence of multidrug-resistant bacterial strains, besides the possibility of being passed along the food chain and may lead to the emergence of novel resistant microorganisms (Morente *et al.*, 2013). On this regard, a variety

of biocide-resistant strains of *Staphylococcus aureus* (Liu *et al.*, 2015), *Escherichia coli* (Capita *et al.*, 2015), and *Salmonella* spp (Molina-Gonzales *et al.*, 2014) have been described in the literature

The Ministry of Agriculture, Livestock, and Supply – Brazil permits the use of plant extracts in the sanitization of equipment and facilities and for the treatment and control of livestock diseases (BRASIL, 2008), through Normative no. 64, September 18, 2008, indicating its concern with the search for alternative products.

In this context, this study aimed to evaluate the antimicrobial activity of *Lippia origanoides* essential oil in the presence of organic matter for disinfection of teats and sanitization of milking utensils.

MATERIAL AND METHODS

Essential oil of *L. origanoides* was obtained from leaves of plants collected in a reservation area of the Institute of Agricultural Sciences of the Federal University of Minas Gerais in Montes Claros, MG - Brazil, of which a voucher specimen is present in the PAMG Herbarium of the Agricultural Research Company of Minas Gerais under record 56526. The essential oil was obtained by steam distillation in a pilot distiller (Linax[®], model D20). As reported by Andrade *et al.* (2014), the extracted oil contain as major constituents carvacrol (29%), o-cymene (25.57%), and thymol (11.50%) and had a minimum inhibitory concentration of 120 µL/mL against strains of *Staphylococcus aureus* ATCC 25923, *Salmonella Choleraesuis* ATCC 10708 and *Escherichia coli* ATCC 8739.

Tests were carried out to determine the disinfectant activity of the oil in the presence of organic matter. The suspension test described by the Ministry of Agriculture, Livestock, and Supply was adopted (BRASIL, 1993). Sterile powdered milk was used as organic matter and bacterial strains of *S. aureus* ATCC 25923, *S. choleraesuis* ATCC 10708, and *E. coli* ATCC 8739 were treated for 5 min. The tests were performed in triplicate and incubated at 37°C and they were repeated for three times.

The test solution contained 600 µL of the essential oil, 40 µL of Tween 80, and sterile distilled water to a final volume of 5 mL, resulting in a final concentration of 120 µL/mL of essential oil. The solution was prepared immediately before use, at each milking.

The tests of effectiveness were executed as proposed by Amaral *et al.* (2004) with modifications. Sixteen healthy cows with no lesions in teats or clinical mastitis were used in the experiment. The right anterior and posterior left teat of each animal

were treated with the sanitizing solution in pre-dipping, and the other left anterior and posterior right teats were used as controls, being cleaned with sterile distilled water. Each group remained with 32 teats.

The disinfectant was sprayed and after 30 s of contact the teats were dried with sterile gauze, with the use of sterile gloves by the milker when performing the procedure. The samples of treated with sanitizer and control teats were collected using sterile swabs previously immersed in a solution of peptone water 0.1 %, by scrubbing them at the extremity of the teats, with rotating movements after 2 min of disinfection and drying them with sterile gauze. The collection area was defined by means of a sterile guide defining a 1-cm² area.

After milking, sixteen sets of teat cups were sanitized with appropriate detergent according to the recommendations of the manufacturer. The right anterior and posterior left teat cups were treated with the sanitizing solution, while the left anterior and posterior right teat cups were used as controls, totaling 32 teat cup rubbers in each group. The sanitizing solution was sprayed and the swab samples were collected after 2 min of application, from a defined 1-cm² area of the internal rubber of the teat cup that is in contact with the teats. The experimental procedures using animals were approved by the Animal Experimentation Ethics Committee of the UFMG, under protocol # 17/2011.

Four polyethylene cans were used to store the milk, and fresh milk was kept for two hours at 4°C. After the milk was removed, the can was sanitized by spraying the detergent according to the recommendations of the manufacturer, and it was kept in contact for 2 min. The procedure was repeated for eight milkings. Samples were collected before and after the application of the product from a 10-cm² area with sterile swabs, totaling 32 samples before and 32 samples after sanitization. The swabs obtained from samples in teat cup and cans were submitted to the same collection procedures mentioned for the teats.

To evaluate the effect of oil in reducing teat microbiota, the teats were evaluated for coagulase-positive *Staphylococcus* and thermotolerant coliforms scores. The teat cups and cans were evaluated for mesophilic aerobes. Microbiological analyzes were performed according to the recommendations of APHA (2001).

The data obtained were submitted to analysis of variance (ANOVA) and Bonferroni's test, using the "Graph Pad Prism" statistical program to compare the concentrations with the control, and between each other.

RESULTS AND DISCUSSION

In the suspension test in the presence of organic matter, essential oil of *L. origanoides* at a concentration of 120 $\mu\text{L/mL}$ showed bactericidal activity against the reference strains *S. aureus* ATCC 25923, *S. choleraesuis* ATCC 10708 and *E. coli* ATCC 8739, after 5 min of exposure. No published data was found regarding this activity of essential oil of *L. origanoides* in the presence of milk or other organic matter. Studies with other essential oils that contain carvacrol, thymol, and other major compounds, showed different results depending on the type of food and bacteria (Raut *et al.*, 2014, Calo *et al.*, 2015). The difficulty in standardizing tests to assess the *in vivo* activity of

essential oils is related to the interactions between specific features of food matrices, characteristics of the microorganism in the presence of food, and chemical groupings of essential oils (Calo *et al.*, 2015).

The disinfectant solution reduced the count of coagulase-positive *Staphylococcus* colonies and thermotolerant coliforms in cow teats (Figure 1) ($p < 0.0001$) and the counts of aerobic mesophilic bacteria in teat cups and milk cans (Figure 2) ($p < 0.0001$) after 5 min of contact, under the conditions of this study. The literature neither provides data on the disinfectant activity of essential oil of *L. origanoides* in teats and milking equipment, nor reports about the contact time required for this purpose.

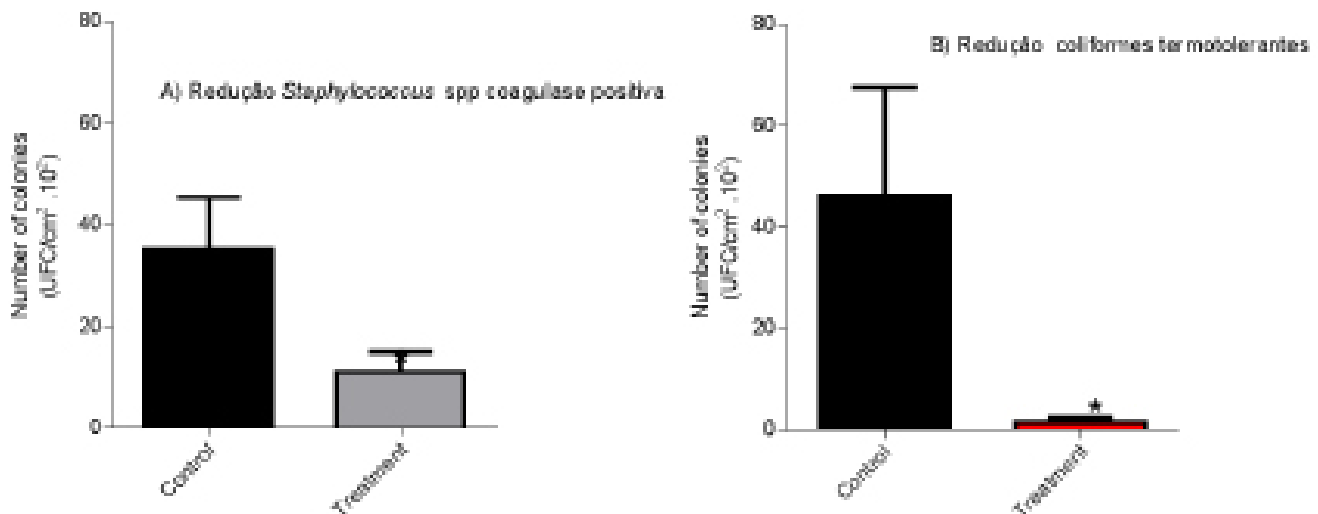


FIGURE 1. Microbial load after treatment with sanitizing solution containing essential oil of *Lippia origanoides*. A) Mean number of *Staphylococcus* spp. coagulase positive colonies on cow teats. B) Mean number of thermotolerant coliforms on cow teats after 5 min. Experimental group with N=16 animals and 32 teats. Asterisk (*) indicates a significant difference between treatments ($p < 0.001$; ANOVA followed by Bonferroni's test).

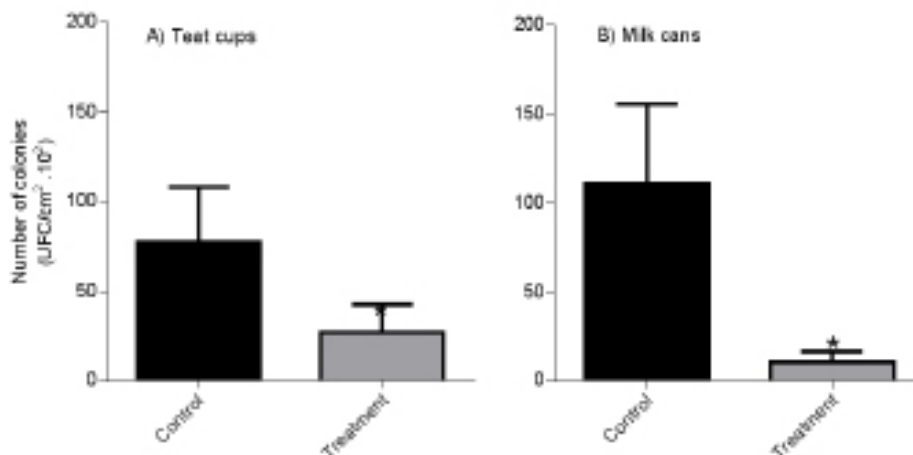


FIGURE 2. Microbial load after treatment with sanitizing solution containing essential oil of *Lippia origanoides*. A) Aerobic mesophilic microorganisms on teat cups and B) Aerobic mesophilic microorganisms in milk cans. N=64 for teat cups and 32 observations in each treatment. N=32 for cans with assessments before and after treatment. Asterisk (*) indicates a significant difference between treatments ($p < 0.001$; ANOVA followed by Bonferroni's test).

The results here obtained are promising, considering the emergence of microorganisms resistant to commonly used biocides due to their extensive use in utensils and equipment sanitizers and the disinfection of teats (Medeiros *et al.*, 2009; Ramalho *et al.*, 2012).

Further studies are required to assess their safety in animals and the impact on the environment and also to determine whether they have a lower risk of microbial resistance or not, and if it is worthy to use it as an alternative to commonly used biocides.

Thus, it could be concluded that essential oil of *L. origanoides* has antibacterial activity in the presence of organic matter and it was able to reduce the bacterial load on the surface of teats and milking utensils.

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REFERENCES

- ALONSO-CALLEJA, C. et al. Adaptation and cross-adaptation of *Escherichia coli* ATCC 12806 to several food-grade biocides. **Food Control**, v. 56, p. 86-94, 2015.
- AMARAL, L. A. et al. Avaliação da eficiência da desinfecção de teteiras e dos tetos no processo de ordenha mecânica de vacas. **Pesquisa Veterinária Brasileira**, v. 24, n. 4, p. 173-177. 2004.
- APHA-AMERICAN PUBLIC HEALTH ASSOCIATION. Compendium of methods for the microbiological examination of foods. 4th Ed. APHA. WASHINGTON, p. 25-35, 2001.
- ANDRADE, V.A. et. al. Antimicrobial activity and acute and chronic toxicity of the essential oil of *Lippia origanoides*. **Pesquisa Veterinária Brasileira**. v. 34, n. 12, p. 1153-1161, 2014.
- BRASIL, Ministério da Agricultura Pecuária e Abastecimento. Portaria n° 101 de 17 de agosto de 1993. **Métodos de Análise Microbiológica para Alimentos**. Diário Oficial da União, Brasília, 17 de agosto de 1993.
- BRASIL, Ministério da Agricultura Pecuária e Abastecimento. Instrução Normativa n° 64 de 18 de dezembro de 2008. Aprova **Regulamento Técnico para os Sistemas Orgânicos de Produção Animal e Vegetal**. Diário Oficial da União, Brasília, 19 de dezembro de 2008.
- CALO, J.R. et al. Essential oils as antimicrobials in food systems - A review. **Food Control**, v. 54, p. 111-119, 2015.
- CAPITA, R. et al. Exposure of *Escherichia coli* ATCC 12806 to sublethal concentrations of food-grade biocides influences its ability to form biofilm, resistance to antimicrobials, and ultrastructure. **Applied and Environmental Microbiology**, v. 80, n. 4, p. 1268-1280, 2014.
- LIU, Q. et al., Frequency of biocide-resistant genes and susceptibility to chlorhexidine in high-level mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MuH MRSA). **Diagnostic Microbiology and Infectious Disease**, v. 82, p. 278-283, 2015.
- MEDEIROS, E.S. et al. Avaliação *in vitro* da eficácia de desinfetantes comerciais utilizados no pré e pós-dipping frente amostras de *Staphylococcus spp.* isoladas de mastite bovina. **Pesquisa Veterinária Brasileira** v. 29, n. 1, p. 71-75, 2009.
- MOLINA-GONZÁLEZ, D et al. Effect of sub-lethal concentrations of biocides on the susceptibility to antibiotics of multi-drug resistant *Salmonella enterica* strains. **Food Control**, v. 40, p. 329-334, 2014.
- RAMALHO, A.C.; SOARES, K.D.A.; SILVA, D.F. *et al.* Eficácia *in vitro* de desinfetantes comerciais utilizados no pré e pós-dipping frente a *Staphylococcus spp.* isolados em rebanhos leiteiros. **Pesquisa Veterinária Brasileira** v. 32, n. 12, p. 1285-1288, 2012.
- RAUT J.S.; KARUPPAYIL S.M. A status review on the medicinal properties of essential oils. **Industrial Crops and Products**. v 62, p. 250-264, 2014.