PHYTOTHERAPIC AND HOMEOPATHIC *In vitro* EFFECTIVENESS OF *Ruta graveolens* TO CONTROL TICKS

EFICÁCIA IN VITRO DE *Ruta graveolens*, NAS FORMAS FITOTERÁPICA E HOMEOPÁTICA, PARA O CONTROLE DE CARRAPATOS

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SUMMARY

The main goal of this research was to test *in vitro* effectiveness of the active principles of the medicinal plant Rue (*Ruta graveolens*), as an alternative way to control ixodid ticks on domestic animals in Campos dos Goytacazes county, Rio de Janeiro. Both phytotherapic and homeopathic in low Hahnemannian`s dinamization forms were tested. The research was performed in the Setor de Parasitologia, Laboratório de Sanidade Animal, Hospital Veterinário of Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). Wild engorged females of *Rhipicephalus (Boophilus) microplus* and *Amblyomma cajennense* collected from naturally infested animals were used. The ticks were divided into groups of ten individuals and, for each group, three different concentrations of both phytotherapic distillation and homephatic dinamization were used, according to WARRC`s (1997) protocol. The phytotherapic product was not effective to control any of the two tick species, while the homeopathic product was effective to control engorged females of *R. (B.) microplus*, reaching 81.81% of unhitched eggs at the highest concentration level, which allows the product to go on the market according to government agencies.

KEY-WORDS: Ixodid ticks. Phytotherapy. Homeopathy. *Ruta graveolens*.

RESUMO

O objetivo desta pesquisa foi testar *in vitro* a eficácia dos princípios ativos originários da planta medicinal Arruda (*Ruta graveolens*), como meio alternativo para o controle de carrapatos ixodídeos em animais domésticos do município de Campos dos Goytacazes, estado do Rio de Janeiro. O trabalho testou tanto o produto fitoterápico quanto o homeopático em baixas dinamizações hahnemmanianas. O experimento foi realizado no Setor de Parasitologia, Laboratório de Sanidade Animal, Hospital Veterinário, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). Foram utilizadas fêmeas ingurgitadas (teleógenas) de *Rhipicephalus (Boophilus) microplus* e *Amblyomma cajennense* originárias de cepas selvagens obtidas de animais naturalmente infestados. Os carrapatos foram separados em grupos de dez e, para cada grupo, foram utilizadas três concentrações diferentes, tanto do extrato fitoterápico quanto da dinamização homeopática, o experimento seguiu o protocolo da WARRC (1997). O produto fitoterápico não demonstrou eficiência no controle de nenhum dos dois carrapatos, enquanto que o produto homeopático obteve sucesso no de teleógenas de *R. (B.) microplus,* atingindo 81,81% de controle de eclodibilidade dos ovos na maior diluição, o que torna o produto aceito para comercialização, segundo as normas dos órgãos governamentais.

PALAVRAS-CHAVE: Carrapatos ixodídeos. Fitoterapia. Homeopatia. *Ruta graveolens*.

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INTRODUCTION

The active ingredients of medicinal plants have been commonly used to control ectoparasites for centuries by the Chinese, Indians, Egyptians and indigenous people. The indigenous people called "Intelligent Spirit" what we now call the active ingredient (MORS et al., 2000; ARAÚJO, 2008).

According to Vattimo & Silva (2011), the World Health Organization (WHO) recommends descriptions and detailed studies of the so-called medicinal plants as determined in Resolution 31.33. The 40ª MAS in 1987, reaffirms these recommendations through Resolution 40.33, which determines that all WHO member countries should initiate study programs for identification, evaluation, preparation, cultivation and conservation of medicinal plants used in traditional medicine and to develop the quality control of drugs derived from traditional medicinal plants, by adopting modern techniques and new practices in their manufacture, and standardizing the products as well (REZENDE & COCCO, 2002; BRASIL, 2006).

Ticks are the vectors of very important diseases from the view point of veterinary and public health, such as: babesiosis, borreliosis and Spotted Fever. Diseases that may lead to death of both humans and animals (MASSARD & FONSECA, 2004; SILVA, 2009; SANTOS et al., 2010). With developing resistance against anti-parasitic drugs, primarily acaricides, the industry has constantly invested in research of new chemical pesticides. One solution for the problems arising from the constant use of traditional medicine is the use of non-conventional therapies such as phytotherapy, homeopathy and acupuncture. Brazil would benefit, especially in foreign markets, if products of animal origin were obtained from organic production, that is, totally free of chemical residues.

It is, therefore, justified to conduct a research to extend to the Veterinary area, the use of active ingredients of medicinal plants to prove scientifically what is already known empirically, the healing power of plants and their ability to maintain the environmental and organic equilibrium.

The objective of this research is to test phytotherapic and homeopathic products to control ixodid ticks that are important threats for Veterinary and Public Health, for this purpose, we selected the medicinal plant Rue (*Ruta graveolens*) since it is empirically indicated to repel insects, mites, ticks and lice.

Rue belongs to Class Dicotyledoneae, Order Rutales and Family Rutaceae. It is popularly known as arruda-fedorenta, arruda-doméstica, arruda-dos-jardins, ruta-de-cheiro-forte and ruda. This subshrub is widely grown in gardens around the world due to its strongly scented leaves. The dried powdered leaves can also be used to treat worm infestation and Leishmaniasis (PEREIRA, 1953; MANVILLE, 1995; OLIVEIRA, 2006).

Its main chemical components are alkaloids, free salicylic acid, methyl-n-nonyl alcohol and its esters combined with acetic acids, bergapten, chalepensin, cineole, kokusaginine, coumarin derivatives, skimmianine, acid methyl ether, phenols, flavonoids, furocoumarin, graveliferona, hesperidin, heterosides anthocyanin, hydrocarbons, hibalactona (root), lactones, limonene (roots mainly), resinous materials and peptic methylnonyl ketone, volatile oils, essential oil (0.07 to 0.09%), pinene, -pipene, psoralens, quercetin, ribalinidin, rubalinidin, rutacridone, rutalidin, rutalinium, rutamarin, rutamin, rutaretin, rutin, methyl salicylate, xanthotoxin (SIMÕES et al., 1999).

MATERIAL AND METHODS

The experiment was performed in the Setor de Parasitologia, Laboratório de Sanidade Animal, Hospital Veterinário, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF).

We used as biological material, extracts of active ingredients of Rue (*R. graveolens*) and the sixth centesimal (C6) homeopathic dynamization**. *Both were acquired commercially from a phytotherapic and homeopathic pharmacy*** (Dr. Renato de Faria®), located in the city of Rio de Janeiro. This plant was chosen based on the action that these active ingredients have on parasitism in humans as cited by Simões et al. (1999).

We used *Rhipicephalus (Boophilus) microplus* and *Amblyomma cajennense* engorged females (teleogines), originating from wild strains that parasitize dairy cattle reared at Escola Técnica Estadual Agrícola (ETEA), and horses reared on a farm, both located in Campos dos Goytacazes county, northern region of Rio de Janeiro state. Ticks were collected homogeneously by the studied species in a single day of July, 2010. It should be noted that in this experiment, ticks were collected from naturally infected animals (WSPA, 2007).

The *in vitro* evaluation was adapted from Drummond et al. (1973), a work cited by the World Acaricide Resistance Reference Center (WARRC, 1997) of the Food and Agriculture Organization (FAO).

The engorged females were collected either from naturally infected animals that had remained 30 days without acaricide or from the stall floors. They were separated in three groups of 10 each that received phytotherapic or homeopathic treatment. Each group received different concentrations: group 1, 100 mL of the active ingredient; group 2, 50 mL of the active ingredient x 50 mL of distilled water; and group 3, 10 mL of the active ingredient x 90 mL of distilled water; while the control group was bathed with distilled water only. The sequence of use started from the highest concentration of the active ingredient. The two first treatments were simultaneously done in replicate except for the control group.

Each tick group was weighed on an analytical scale, and further transferred to a beaker containing the

previously prepared solution to be tested. The beaker was stirred constantly for five minutes. After that, the ticks were placed on filter paper (15 cm^2) to remove excess of product, and then transferred to Petri dishes (100 mm x 20 mm height). After labeling, they were transferred to the BOD incubator at 28ºC and 80% relative humidity. After oviposition, the depositions of each group of female were weighed, transferred to previously adapted plastic syringes, labeled and taken back to the same incubator.

After 30 days we counted the eggs that hatched using a stereoscopic microscope (with magnifying glass), and the control group as reference. The reproductive efficiency (RE) was calculated by the following equation:

$$RE = \frac{\text{egg weights}}{\text{female weights}} \times \%\ \text{hatched} \times 20.000$$

After RE was calculated, control percentage was calculated according to the following equation, using the arithmetic average of the three repetitions:

$$\%\ \text{control} = \frac{\text{RE (non-treated)} - \text{RE (treated)}}{\text{RE (non-treated)}} \times 100$$

This study meets the requirements for research with animals, is consistent with the ethical principles and practical use of animals in experiments and it was approved by the Ethics Committee of Universidade Federal Rural do Rio de Janeiro, protocol number 23083.005374/2011-13.

RESULTS

The WHO establishes that acaricide treatments are effective when average mortality is higher than 80% (likely status of vectors to the substance) and ineffective below 80% (resistance status) (WHO, 2007). Likewise, the Ministério da Agricultura Pecuária e Abastecimento (MAPA) provides for the registration of the acaricide, ixodid mortality of at least 75% at the recommended dosage (MINISTÉRIO DA AGRICULTURA, 2007).

In the phytotherapic treatment with the active ingredient of *R. graveolens*, the highest mean percentage of hatchability of *A. cajennense* eggs was 32.48% without dilution. For *R. (B.) microplus*, the highest mean percentage was 21.42% for the highest dilution (Table 1). Thus the phytotherapic treatment was not an effective acaricide for either tick species, according to WHO and MAPA.

In the homeopathic treatment using the active ingredient of *R. graveolens* dynamized to the sixth centesimal (C6), the highest mean percentage of hatchability of *A. cajennense* eggs was 50.41% without dilution. Therefore, the product failed as an effective acaricide according to WHO and MAPA (Table 2). Finally, the highest mean percentage of hatchability of *R.* (*B.) microplus* eggs reached was 81.81% at the highest dilution, which makes this product commercially acceptable, according to the regulating agencies (Table 2).

DISCUSSION

In this experiment, rue used as a herbal plant extract was not effective to control hatchability of *R.* (*B.) microplus* eggs. This result corroborates the findings of Clemente et al. (2007), who reported that *R. graveolens* plant extract was not an effective acaricide in any of the four concentrations studied (6.25%, 12.5%, 25% and 50%), for both larvae and females. Likewise, Nogueira et al. (2007) concluded that aqueous extracts of several plants, including rue were not an effective repellent against *R.* (*B.) microplus* tick larvae.

It was observed that the highest rates of hatchability control of *R.* (*B.) microplus* eggs occurred when the product was used in higher dilutions. These results suggested a positive correlation between higher hatchability control rates and higher dilutions. This hypothesis led us to choose to test rue as a homeopathic treatment at the C6 dynamization. The 81.81% hatchability percentage control obtained against eggs of *R.* (*B.) microplus* proved its efficiency as acaricide since the value is above the standard recommended by WHO and MAPA and can, therefore, be commercialized as such. Souza et al. (2007) also concluded that a homeopathic product was effective and significant to control ticks in cattle.

CONCLUSION

R. (*B.) microplus* treated with a high dilution (C6) of the homeopathic product resulted in 81.81%, a high percentage of egg hatchability control that makes it feasible for commercial use according to the regulating agencies. However, the homeopathic product was not effective to control egg hatchability of *A. cajennense*, since mean mortality rate of 50.41% was below the values recommended by WHO and MAPA for commercialization. The results of this research showed that it was a great choice to replace the herbal extract with the homeopathic product, since it was shown that many dynamizations potentiate the effect of the active ingredient of the medicinal plant. Further research is suggested mainly to elucidate the selection of molecules of the active ingredient of phytotherapic rue

Table 1 – Mean and individual percentage of hatchability control of *Amblyomma cajennense* and *Rhipicephalus (Boophilus) microplus* eggs obtained in the three repetitions at three concentrations of *Ruta graveolens* phytotherapic treatments.

Concentrations	Control (%)			Mean (%)
	1ª repetition	2ª repetition	3ª repetition	
Amblyomma cajennense				
100 mL phytotherapic product	31.15	35.47	30.82	32.48
50 mL phytotherapic product / 50 mL H_2O	18.64	17.45	19.67	18.58
10 mL phytotherapic product / 90 mL H_2O	13.58	14.97	13.21	13.92
Rhipicephalus (Boophilus) microplus				
100 mL phytotherapic product	11.2	6.8	7.2	8.4
50 mL phytotherapic product / 50 mL H_2O	9.23	8.15	12.05	9.81
10 mL phytotherapic product / 90 mL H_2O	21.07	23.71	19.48	21.42

Table 2 - Mean and individual percentage of hatchability control of *Amblyomma cajennense* and *Rhipicephalus (Boophilus) microplus* eggs obtained in the three repetitions at three concentrations of *Ruta graveolens* (C6) homeopathic treatment.

Concentrations	Control (%)			Mean (%)
	1ª repetition	2ª repetition	3ª repetition	
Amblyomma cajennense				
100 mL C6	48.68	57.23	45.32	50.41
50 ml C6/50 mL H_2O	47.57	40.12	42.33	43.34
10 ml C6/90 mL H_2O	39.23	43.56	45.72	42.83
Rhipicephalus (Boophilus) microplus				
100 ml C6	13.47	15.23	14.89	14.53
50 ml C6/50 mL H_2O	15.87	13.35	17.25	14.59
10 ml C6/90 mL H_2O	87.62	75.46	82.36	81.81

that have acaricidal effect. Findings like this are good for the country, since the bases of acaricide sold commercially are mostly foreign and costly for the producer. Furthermore, it is expected that the use of medicinal plants for the control of ticks will also enhance the quality of life, especially by minimizing the chemical residues found in meat and milk products.

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