DEVELOPMENT OF SPRAY FORMULATIONS CONTAINING EUGENOL AND CARVACROL FOR FLEA AND TICK CONTROL

Jesus, I. L. R.[1*]; Miranda, F. R.[1]; Campos, D. R.[1]; Cid, Y. P.[2]

[1]Programa de Pós-Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, RJ, Brasil. [2]Docente do Departamento de Ciência Farmacêuticas (DCFar), Universidade Federal Rural do Rio de Janeiro UFRRJ), Seropédica, RJ, Brasil.

**raquellingrid@gmail.com*

Introduction

The incorrect and indiscriminate use of acaricides and insecticides by tutors, often without monitoring by a veterinarian, has contributed to the development of resistance of many parasites to treatment [1,3,5]. These synthetic products can have high toxicity, to which animals, tutors and the environment are exposed [3]. Therefore, efforts have been made to develop products with selective toxicity that act on the parasite in question, but that are less aggressive to animals and the environment. Thus, natural products that are already widely used in agriculture for insect control, emerge as an alternative and promising treatment for controlling these parasites, such as essential oils (EO). Essential oils are natural substances obtained from plants that may have insecticidal and acaricidal activity, and this activity is associated with the major constituent, such as eugenol and carvacrol. Eugenol is a phenylpropanoid and carvacrol is a monoterpene, found as major constituents of several essential oils, such as clove (*Syzygium aromaticum*) and oregano (*Origanum vulgare*) essential oils, respectively, both having antiparasitic properties already described against several groups of parasites [4].

The objective of the present work was to develop spray formulations containing 10% eugenol, 10% carvacrol and the combination of both in the same formulation at 10% each, characterize them, perform stability tests and evaluate their residual efficacy *in vitro* against adult cat fleas (*Ctenocephalides felis felis*) and tick *Rhipicephalus sanguineus.*

Material and Methods

Three formulations were developed in the form of a spray, containing as active: 10% eugenol, 10% carvacrol and a mixture of both at 10% each; antioxidant; penetration agent; surfactants; acidifying and chelating agent; pH 7.0 buffer and vehicle. The formulations were conditioned in amber and transparent bottles for later physical-chemical characterization and preliminary and accelerated stability studies, such as pH evaluation, organoleptic characteristics, freezing/heating cycle, centrifugation and weight variation for five days.

For the *in vitro* bioassays, the filter paper impregnation method was used, in which a 10 cm^2 filter paper strip is impregnated with 200µL of the formulation and placed in tubes containing five couples of unfed adult fleas, aged 14 days [2]. For the *in vitro* bioassay with ticks, the 63.75 cm^2 filter paper was impregnated with 670µL and five couples of unfed adult *R. sanguineus*, aged 14 days, were deposited. Residual efficacy was evaluated every 24 hours, counting live and dead individuals, replacing them with new couples for both ectoparasites until no more mortality was observed. All *in vitro* bioassays were performed in sextuplicate and concomitantly with placebo formulations. Mortality is calculated according to the Abott formula, described below:

$$\text{Mortality } (\%) = \frac{\text{mortality}_{\text{control group}} - \text{mortality}_{\text{treated group}}}{100 - \text{mortality}_{\text{control group}}} * 100$$

Results and Discussion

Liquid, translucent, slightly yellow formulations were obtained, with a slightly more intense coloration in the formulation containing the association of actives, without precipitates, remaining unchanged over a month. When subjected to the freezing and heating cycle, a darkening of the formulations was noticed, due to the oxidation of the actives when exposed to high temperatures (40ºC). After centrifugation, the formulations showed no precipitate formation, remaining homogeneous. The formulations had their pH adjusted to adequate values for topical application in animals, whose range is 5.5 -7.0, obtaining an average pH of 6.81 ± 0.06; 6.40 ± 0.06 and 6.61 ± 0.08, for formulations with eugenol, carvacrol and combination of actives, respectively. In general, the best way to stock the formulation is in an amber bottle and in the refrigerator, which ensure less formulation loss and protect the photosensitive active from possible oxidation.

Residual efficacy testing for fleas is still ongoing, but over the course of 30 days, the eugenol containing formulation had 100% mortality for 14 non-consecutive days, reaching 90% on the 30th day of analysis; while the formulation containing carvacrol showed maximum mortality in 5 non-consecutive days, which is 60% to date. However, the formulation containing both showed maximum efficacy on 11 non-consecutive days, remaining at 90% efficacy for one month. While for the tick, the acaricidal activity was 72 hours for all formulations, and the mortality of formulations containing eugenol and with the association of actives decreased from 100% on the first day to 10% on the last day of analysis, while the formulation containing carvacrol reduced from 77% to 10% in the same period.

Conclusion

It is concluded that the spray formulations developed with the actives at 10% have insecticidal activity with residual efficacy greater than 30 days for *C. felis felis*. It can be seen that the formulation an association of actives provides maximum effectiveness for a period longer than the others. In addition, the formulations have potential acaricidal activity against adults of *R. sanguineus*, with adequate and stable characteristics for the proposed purpose.

Acknowledgments

CAPES, CNPq and FAPERJ .

Bibliographic References

[1] COLES, T. B.; DRYDEN, M. W. Insecticide/acaricide resistance in fleas and ticks infesting dogs and cats. Parasites and Vectors, v. 7, n. 1, p. 1–10, 2014.

[2] CONCEIÇÃO, C.L., de Morais, L.A.S., Campos, D.R. et al. Avaliação da Atividade Inseticida de Tomilho, Orégano e Óleos Voláteis de Cássia em Pulga de Gato. Rev. Bras. Farmacogn. 30, 774-779 (2020).

[3] GUPTA, R. C.; DOSS, R. B.; SRIVASTAVA, A.; LALL, R.; SINHA, A. Nutraceticals for control of ticks, fleas and other ectoparasites. Springer Nature Switzerland, p. 625-632, 2019.

[4] LAMBERT, M. M. et al. Activity of Syzygium aromaticumessential oil and its main constituent eugenol in the inhibition of the development of Ctenocephalides felis felisand the control of adults. Veterinary Parasitology, v. 282, n. April, p. 109126, 2020.

[5] RUST, M. K. Insecticide resistance in fleas. Insects, v. 7, n. 1, 2016.