In vitro and in vivo acaricide action of a juvenoid analogue produced from the chemical modification of Eucalyptus citriodora essential oil on Rhipicephalus (Boophilus) microplus*

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Introduction

The cattle tick *R.* (*B.*) microplus cause annual losses of 2 billion dollars in Brazil (Grisi et al., 2002) and plant extracts have been extensively researched for detection of new bioactive. This study aimed to evaluate the acaricide action of *E. citriodora* essential oil, chemically modified, on *R.* (*B.*) microplus.

Material and Methods

The citronellal was converted into *N*-prop-2-inylcitronellylamine by reaction with *N*-propargylamine under microwave irradiation and followed by reduction with NaBH₄. The *N*-prop-2-inylcitronellylamin formation, an analogue of juvenoid, was accompanied by IR analysis, which showed the disappearance of carbonyl aldehyde band of the citronellal and the appearance of the NH band of the amine. In the GC/MS was confirmed the formation of the citronellylamine and checked that the minority components of the oil have not been modified.

Test 1 Immersion Test (Fig. 1): Engorged females collected from cattle were weighed in groups of 10, and held 3 replicates for each concentration (100, 50, 35, 25, 12.5 e 6.25 mg/ml). They were immersed in the modified oil (N7), in the original oil and control for 5 min. and incubated for 18 days. The posture and larval hatchability were then observed (Drummond et al., 1973).









Figure 1. a) Immersion of engorged female on the substances, b) Female drying after immersion, c) Incubation on Petri dishes (27°C, UR>80%), d) Evaluation posture and larval hatchability

Test 2 Larval Packet Test (Fig. 2):The N7 and oil were evaluated in the same concentrations and in 3 replicates in which about 100 larvae were placed on filter paper impregnated and incubated by 24h. The living and dead larvae were then counted (FAO, 1971).









Figure 2. a) R. (B.) microplus larvae colony, b) Incubation of larvae in the packets of impregnated filter papers (27°C, UR-80%), c) and d) Living and dead larvae been counted with the ald of a vacuum nump attached to a pipette.

Test 3 $In\ vivo\ (Fig.\ 3)$: 6 heifers were used in each treatment: negative control (water), positive control (commercial acaricide Triatox), original oil of $E.\ citriodora\ at\ 1.5\%$ and modified oil containing 0.9% of N-prop-2-inylcitronellylamine. Ticks >4.5 mm in size were counted in the right side of the body in 34 animals twice before treatment to form homogeneous groups (D-3 and D-7) and then on D0, D3, D7, D14 and D21. LC_{50} and LC_{90} were obtained by Probit, while the $in\ vivo\ results$ were log transformed and compared with the Tukey test.

Results

The analysis identified that eucalyptus oil was composed by 60% citronellal, that after be modified, was quantitatively converted to N-prop-2-inylcitronellylamine. In the female test we had 0% of efficacy at 100 mg/mL for the original oil, while for the N7 the LC $_{50}$ and LC $_{90}$ were respectively 35.9 and 106.7 mg/mL and, in the larvae test were respectively 8.6 and 12.2 mg/mL for the original oil and 3.3 and 3.8 mg/mL for N7. In the $in\ vivo$ test was not observed significant difference of the N7 in relation to the original oil and negative control until day 21 (Fig. 4). The general averages of ticks from the animals' right side in the four treatments were 34.0, 18.5, 36.4, and 35.5, respectively.



Figure 3. Preparation of the substances to treat cattle by aspersion in the field test.

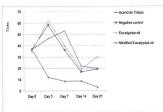


Figure 4. Average number of the engorged females counted in the right side of the cattle body in the four groups on days 0, 3, 7, 14 and 21 after treatment.

Conclusion

The chemical modification demonstrated that the efficacy was not just kept but also improved in vitro, but despite these excellent results, efficacy has not been observed in vivo, perhaps due to the low stability of citronellylamine under the field conditions.

References

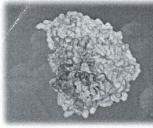
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