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PHYTOCHEMICAL STUDY AND *IN VITRO* ACARICIDAL ACTION OF *Tagetes patula* L. (ASTERACEAE) ETHANOLIC EXTRACT AGAINST ENGORGED FEMALES OF *Rhipicephalus (Boophilus) microplus*

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Abstract: *Tagetes patula* L. (Asteraceae) is an annual plant native to North America and widely disseminated throughout the world. Due to the rich composition of secondary metabolites in its aerial parts [1], there are many studies in the literature reporting its biocide effect against insects [2-4], ticks [5-7] and nematodes [8], increasing interest in the evaluation of this species against endo and ectoparasites. The aim of this study was to test the acaricide potential of ethanolic extract of *T. patula* against engorged females of *Rhipicephalus (Boophilus) microplus*, a tick species responsible for huge financial losses to the Brazilian livestock [9]. Briefly, have been used 10 ticks per group, immersed for 5 minutes in the test solutions. The ethanolic extract was applied at eight concentrations, and the results were expressed by the efficiency of product [10]. All tests were done in triplicate, using ethanol and distilled water as negative controls [11]. For phytochemical analysis, the fractions obtained from the ethanolic extract by column chromatography, were analyzed by HPLC-DAD in exploratory gradient mode. The compounds isolation in two selected fractions was performed through a Luna C18 column (150 x 21.2 mm), in preparative scale, applying the following chromatographic method: [A (33% H_2O) and B (binary mixture: 54% MeOH, 13% ACN), isocratic mode during 20 minutes, flow 20 $mL.min^{-1}$, injection volume 500 µL]. NMR spectra 1 and 2D were acquired in Bruker Fourier 600 spectrometer, using tetramethylsilane as internal reference. Aliquots of the fractions III and V of *T. patula* were analyzed at 5 mg/700 µL of $CDCl_3$. The spectroscopic data for isolated compounds **1**, **2** and **3** agreed with those described in the literature, making it possible to confirm the identification of tremetone (**1**), 5-acetyl-6-hydroxy-2-isopropenyl-2,3-dihydrobenzofuran (**2**) and 6-acetyl-2,2-dimethylchroman-4 -one (**3**), described for the first time in the species, however, already identified in the Asteraceae family [12-14]. The table 1 summarizes the results achieved with the *in vitro* test, highlighting the significant values of inhibition of oviposition and eggs hatching by the higher extract concentrations. By probit analysis, the LC_{50} and LC_{90} were calculated: LC_{50} = 18.6 $mg.mL^{-1}$ (11.0 – 31.4 $mg.mL^{-1}$) and LC_{90} = 106.8 $mg.mL^{-1}$ (56.1 – 364.2 $mg.mL^{-1}$). These results are quite promising, and complementary *in vivo* assays can confirm the use of *T. patula* extract as an option to synthetic acaricides, since it is demonstrated the absence of toxicity for use in cattle.

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Table 1. Parameters evaluated in the Adult Immersion Test (AIT) employing ethanolic extract of *Tagetes patula* against engorged females of *Rhipicephalus (Boophilus) microplus*.

Tp_{EtOH} (mg.mL^{-1})	M_{Ticks} (g)	M_{Eggs} (g)	Eclosion (%)	EP (%)
200	2.68 (± 0.12)	0.02 (± 0.03)	10 (± 17.32)	99.2^{a} (± 1.3)
100	2.56 (± 0.03)	0.24 (± 0.36)	10 (± 17.32)	91.7^{a,b} (± 14.4)
50	2.53 (± 0.03)	0.29 (± 0.27)	21.67 (± 24.66)	86.4^{a,b} (± 20.9)
25	2.55 (± 0.03)	0.63 (± 0.12)	66.67 (± 15.28)	47.2^{b,c} (± 20.7)
12.5	2.52 (± 0.005)	0.87 (± 0.10)	71.67 (± 18.93)	20.5^{c,d} (± 28.4)
6.25	2.52 (± 0.01)	0.84 (± 0.07)	76.67 (± 7.64)	18.3^{c,d} (± 15.2)
3.12	2.61 (± 0.97)	1.01 (± 0.38)	71.67 (± 31.97)	12.2^{c,d} (± 12.0)
1.56	2.53 (± 0.01)	0.84 (± 0.06)	85.00 (± 5.00)	10.6^{c,d} (± 8.4)
C- (EtOH)	2.24 (± 0.02)	0.92 (± 0.02)	85.00 (± 13.23)	0.3^{e} (± 0.5)

*Tp_{EtOH} = ethanolic extract of *T. patula*; C- = negative control; M_{Ticks} = mass of engorged ticks (mean value ± standard deviation); M_{Eggs} = mass of eggs laid (mean value ± standard deviation); EP = efficacy of the product. Means with the same superscript letter are not significantly different by Tukey Test ($p \leq 0.05$).

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