



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% H₂O) 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 CDCl₃. 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 LC₅₀ and LC₉₀ were calculated: LC₅₀ = 18.6 mg.mL⁻¹ (11.0 – 31.4 mg.mL⁻¹) and LC₉₀ = 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.



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 ^{ab} (± 14.4)
50	2.53 (± 0.03)	0.29 (± 0.27)	21.67 (± 24.66)	86.4 ^{ab} (± 20.9)
25	2.55 (± 0.03)	0.63 (± 0.12)	66.67 (± 15.28)	47.2 ^{bc} (± 20.7)
12.5	2.52 (± 0.005)	0.87 (± 0.10)	71.67 (± 18.93)	20.5 ^{cd} (± 28.4)
6.25	2.52 (± 0.01)	0.84 (± 0.07)	76.67 (± 7.64)	18.3 ^{cd} (± 15.2)
3.12	2.61 (± 0.97)	1.01 (± 0.38)	71.67 (± 31.97)	12.2 ^{cd} (± 12.0)
1.56	2.53 (± 0.01)	0.84 (± 0.06)	85.00 (± 5.00)	10.6 ^{cd} (± 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 ≤ 0.05).

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