

## Scientific Paper

*In vitro* acaricidal activity of the oil from *Jatropha curcas* L. in engorged females of *Rhipicephalus (Boophilus) microplus*

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**Abstract**

In order to evaluate the *in vitro* efficacy of the oil from the *Jatropha curcas* L. fruit in engorged females of *Rhipicephalus (Boophilus) microplus* (certified Cayo Coco strain), an immersion test of adult ticks was conducted, based on a completely randomized design and nine treatments: negative control of Tween-80 at 5, 10, 15 and 25 %; positive control with Butox® (Deltametrina) and different concentrations of *J. curcas* oil: 5, 10, 15 and 25 %. The hatching reduction, estimated reproduction and acaricidal efficacy were determined. Through a variance analysis the efficacy of the treatments was determined and by a Probit analysis, the mean effective concentration (EC<sub>50</sub>) and maximum effective concentration (EC<sub>90</sub>). The oil significantly inhibited ( $p < 0,05$ ) the egg hatching percentage, with values of 9,74; 11,40; 11,95 and 13,75 % for the concentrations of 5, 10, 15 and 25 %, respectively. No significant effects were observed in the egg weight, but they were appreciated in the estimated reproduction ( $p < 0,05$ ) for the experimental treatments with regards to the negative control. The oil of the *J. curcas* fruit showed marked efficacy as acaricide in the control of *R. (B.) microplus*, with values higher than 91 %; the best results were found with the lowest concentration (5 %). The dose-response curve showed an EC<sub>50</sub> of 88,90 mg/mL, while the EC<sub>90</sub> was 37,22 mg/mL. It is concluded that the *J. curcas* oil has *in vitro* acaricidal activity.

**Keywords:** hatching, ticks, eggs

**Introduction**

Ticks have become adapted to most of the terrestrial niches in the planet and have become specialized in feeding from blood of mammals, birds and reptiles. Although different species are present in different ecological regions, *Rhipicephalus (Boophilus) microplus* is one of the most important for livestock production in tropical and subtropical regions of the planet (Cortés, 2010; Fernandes *et al.*, 2012; Del Castillo *et al.*, 2016).

On the other hand, the current scenarios are characterized by a sustained economic crisis of the farming sector and increasingly regionalized, competitive and demanding markets. In this economic-productive context, if a drastic change does not occur in the control approach, a progressive increase of multiple resistance cases in different species-genera of parasites that affect animal production should be expected (Domínguez *et al.*, 2010; Amaral *et al.*, 2011; Fernández-Salas *et al.*, 2012; Grisi *et al.*, 2014).

During recent years, there is in the world great interest in researching the presence of pharmacological principles in those plants which, due to popular

tradition, are acknowledged in ethnobotany studies as antiparasitic and also used by farmers. The extracts of these plants show higher safety, low cost and efficacy; besides, they do not damage the ecosystems or human health (Silva *et al.*, 2011; Isea *et al.*, 2013; Rodríguez-Vivas *et al.*, 2014).

In several tropical countries, many species that show insecticidal actions are acknowledged; among them is *Jatropha curcas* L., considered the agroenergy crop of the future. Studies with different parts of the plant report the following effects: anticonceptive, antimycotic, acaricidal, anti-inflammatory, healing and coagulant (Carrasco-Rueda *et al.*, 2013). In addition, its seeds are toxic, for which their oil is not edible and does not compete with human feeding.

Considering the above-mentioned facts, the objective of this study was to evaluate the *in vitro* efficacy of the oil from the *J. curcas* L. fruits in engorged females of *R. (B.) microplus*.

**Materials and Methods**

**Location.** The research was conducted in the parasitology laboratory of the Pastures and Forages

Research Station Indio Hatuey (Perico, Matanzas, Cuba), and in the animal parasitology laboratory of the National Center of Agricultural Health –CENSA, for its initials in Spanish– (San José de las Lajas, Mayabeque, Cuba).

**Obtainment of the biological material.** The Cayo Coco strain of *R. (B.) microplus*, obtained by the National Laboratory of Veterinary Parasitology (San Antonio de los Baños, Artemisa, Cuba) was used.

For obtaining the biological material the methodology described in Resolution 48, 1997, of the Ministry of Agriculture, Farming and Supply of Brazil, was used, which describes the artificial infestation of donor animals under isolation conditions. Once collected, the ticks were washed with chlorinated distilled water at 1 %, during one minute, and dried with paper towels. The engorged female ticks (full or ingurgitated females) that fulfilled the characteristics recommended by Farias *et al.* (2007): normal appearance and motility, whole body and maximum ingurgitation, were selected.

The oil was obtained from the mature fruits, dried under sunlight and unshelled; afterwards, the seeds were pressed, according to the methodology described by Sotolongo *et al.* (2007).

**Treatments and experimental design.** A completely randomized design was used with twelve replications for each treatment and ten ticks for each replica, for a total of 120 ticks per treatment.

The treatments were the following:

- T1: Butox® –Deltametrina– (positive control)
- T2: Tween-80 5 % (negative control)
- T3: Tween-80 10 % (negative control)
- T4: Tween-80 15 % (negative control)
- T5: Tween-80 25 % (negative control)
- T6: physic nut oil 5 % + Tween-80
- T7: physic nut oil 10 % + Tween-80
- T8: physic nut oil 15 % + Tween-80
- T9: physic nut oil 25 % + Tween-80

**Experimental procedure.** The immersion test of engorged *R. (B.) microplus* females was used, according to the efficacy techniques described by Drummond *et al.* (1973), modified by Chagas *et al.* (2012).

After being collected, the ticks were individually weighed and grouped according to their weight (between 0,20 and 0,29 g). They were put in test tubes, numbered and identified by treatment, number of replica and date of starting the experiment. To each test tube 5 mL of the solution to be evaluated were added. After five minutes the liquid was drained

through a colander and the ticks were placed on paper napkins to remove the residual liquid, and then fixed on adherent paper in previously identified Petri dishes.

These Petri dishes were placed in incubator at 27 °C and with relative humidity of 80 %. After the egg production phase, the eggs were independently collected and deposited in test tubes identified with the same key. The tubes were covered with cotton swabs and placed in the incubator during 18 days until the end of the hatching process.

**Experimental measurements.** During the experiment the initial weight of the engorged females, the initial and final day of egg production, the number of dead ticks and the weight of the eggs, were recorded. With these measurements the following indicators were determined: duration of the egg production period, mortality rate of the engorged females, hatching percentage, hatching period and control or efficacy percentage. The studied variables were transformed into: hatching reduction, estimated reproduction and acaricidal efficacy.

Hatching reduction (%) (HR)

$$HR = \frac{[(\text{mean hatching of the control group} - \text{mean hatching of the treated group}) / \text{mean hatching}] \times 100}{[RP1]}$$

Estimated reproduction (ER)

$$ER = \frac{[(\text{weight of the egg mass} \times \text{hatching} (\%)) / \text{weight of the engorged female}] \times 20\,000}{[RP1]}$$

Efficacy of the acaricide (EA) (%)

$$EA = \frac{[(HR \text{ of the control group} - HR \text{ of the treated group}) / HR \text{ of the control group}] \times 100}{[RP1]}$$

**Statistical analysis.** The data were recorded in spreadsheets of Microsoft® Excel® to perform their respective analyses. A variance analysis was made in order to determine the differences among the means. The percentage data (efficacy) were transformed into to achieve a distribution close to normal; the results are shown with the arithmetic means and a significance level of 5 % was used.

Duncan's multiple range comparison test was used for establishing the differences among means, through the statistical pack IBM® SPSS® Statistics version 22. The calculatin of the effective concentrations (EC<sub>50</sub> and EC<sub>90</sub>) was made through a Probit analysis with the statistical pack SAS® 9.3.

## Results and Discussion

The oil from the *J. curcas* L. fruit significantly inhibited ( $p < 0,05$ ) the egg hatching percentage of

the treated engorged females of *R. (B.) microplus*, with values of 9,74; 11,40; 11,95 and 13,75 % for the oil concentrations of 5, 10, 15 and 25 %, respectively (table 1). On the other hand, the negative control (solvent Tween-80) did not interfere significantly on egg hatching, because hatching percentages higher than 88 % were recorded for the same concentrations, which proved that the solvent used can be utilized for this type of essays.

In this sense, Chagas (2008) proved that the solvents with low molecular weight and little viscosity do not interfere in the efficacy of products for biological tests with *R. (B.) microplus*, and can be used in concentrations lower than 76 %.

On the other hand, it could be appreciated that the oil did not have a significant effect ( $p < 0,05$ ) on the egg weight. Nevertheless, it was observed that the estimated reproduction (quantity of laid eggs of the treated engorged females) was significantly affected ( $p < 0,05$ ) in the treatments that included oil from the *J. curcas* L. fruit, with regards to the control group.

The best results corresponded to the lowest concentration, which could be related to better

emulsion of the oil at that concentration, allowing higher adhesion in the cuticle of the engorged female tick, facilitating its bioavailability and penetration. According to Chagas *et al.* (2012) from this phase effects are observed on the reproductive system of the engorged female tick with the subsequent inhibition in the egg production percentage and the egg fertility. On the other hand, Castillo *et al.* (2016) stated that the oils can act as inhibitors of the enzymatic activity of the digestive tract, affecting the feeding function.

Table 2 shows the results of the efficacy of the oil from the *J. curcas* L. fruit on the engorged females of *R. (B.) microplus*. Significant differences were observed ( $p < 0,05$ ) among the treatments and the concentrations (96,06; 94,65; 91,54 and 91,04 % for 5, 10, 15 and 25 %, respectively). The negative control, as it was to be expected, showed null efficacy; while the efficacy of Butox® was 99,49 %.

These results are similar to the ones reported by Santos and Vogel (2012), when evaluating the *in vitro* effects of essential oils from *Cymbopogon citratus*; and by Farias *et al.* (2007), who found *in vitro* efficacy of the *Carapa guianensis* oil (between

Table 1. Effect of the oil from the *J. curcas* L. fruit on the egg weight, hatching percentage and estimated reproduction of engorged *R. (B.) microplus* females.

Treatment	%	Egg weight (g)	% of hatching	ER (e/g)
Butox®		0,005	0 <sup>f</sup>	0 <sup>a</sup>
Tween-80	5	1,349	91,12 <sup>ab</sup>	698 285 <sup>ed</sup>
	10	1,352	90,50 <sup>b</sup>	727 415 <sup>c</sup>
	15	1,318	89,62 <sup>b</sup>	687 404 <sup>d</sup>
	25	1,269	88,63 <sup>b</sup>	675 530 <sup>d</sup>
<i>J. curcas</i> oil	5	0,415	9,74 <sup>c</sup>	24 947 <sup>b</sup>
	10	0,521	11,40 <sup>d</sup>	35 587 <sup>b</sup>
	15	0,736	11,95 <sup>d</sup>	52 807 <sup>c</sup>
	25	0,755	13,75 <sup>c</sup>	59 051 <sup>c</sup>

Values with different superscripts in the same column differ at  $p < 0,05$ .

Table 2. Efficacy of the oil from the *J. curcas* L. fruit on the engorged females of *R. (B.) microplus*.

Treatment	Butox®	Tween-80	<i>J. curcas</i> oil
Concentration %	Efficacy (%)		
5	99,49 <sup>d</sup> ± 0,09	0,00 * ± 0,00	96,06 <sup>c</sup> ± 1,24
10		0,00 * ± 0,00	94,65 <sup>c</sup> ± 0,92
15		0,00 * ± 0,00	91,54 <sup>b</sup> ± 1,70
25		0,00 * ± 0,00	91,04 <sup>b</sup> ± 1,75

Values with different superscripts differ at  $p < 0,05$ .

70 and 100 %) for all the evaluated dilutions, with a mortality rate in the engorged female ticks between the second and third day post-treatment. The eggs were infertile, and the best results were found in the oil concentration at 5 %.

On the other hand, Martínez-Velázquez *et al.* (2011a; 2011b) reported acaricidal effects with efficacy values between 85 and 100 %, for this tick species, when using essential oils extracted from *Cuminum cyminum*, *Pimenta dioica*, *Lippia graveolens*, *Rosmarinus officinalis* and *Allium sativum*.

Although there are no reports in literature about the acaricidal properties of the oil from the *J. curcas* L. fruit, in a study Rugama (2003) evaluated three glycerol concentrations –obtained through the processes of this oil– for the control of the tick *R. (B.) microplus*, and found significant results for the concentrations of 20 and 30 %, with values between 90 and 95 % of efficacy 60 h after applying the product.

Figure 1 shows the dose-response curve of the oil in the treated engorged female ticks. This allowed to obtain an  $EC_{50}$  of 88,90 mg/mL and an  $EC_{90}$  of 37,22 mg/mL. These values are lower than the ones reported by Heimerdinger *et al.* (2014), who obtained an  $EC_{50}$  of 91,8 mg/mL; however, they are similar to the ones reported by Chagas *et al.* (2012) when essential oils from *Carapa guianensis*, *Cymbopogon martinii* and *Cymbopogon schoenanthus* were evaluated on adults of this species.

Several *in vitro* studies have proven the potential of plants, their oils and other substances isolated for the control of the tick *R. (B.) microplus* (Chagas *et al.*, 2012; Isea *et al.*, 2013). The oils are complex mixtures that contain tens or even hundreds of substances with wide chemical activity, and their efficacy

can vary depending on multiple factors (Fourie *et al.*, 2013). Yet, the low dilution used in this study suggests that the concentration of active principles in the oil can be higher than the one found in aqueous extracts, hence the importance of continuing these studies.

## Conclusions

The oil from the *J. curcas* L. fruit showed marked *in vitro* acaricidal efficacy, as well as on egg hatching and estimated reproduction, with an effective concentration  $LC_{50}$  of 88,90 mg/mL and  $LC_{90}$  of 37,22 mg/mL.

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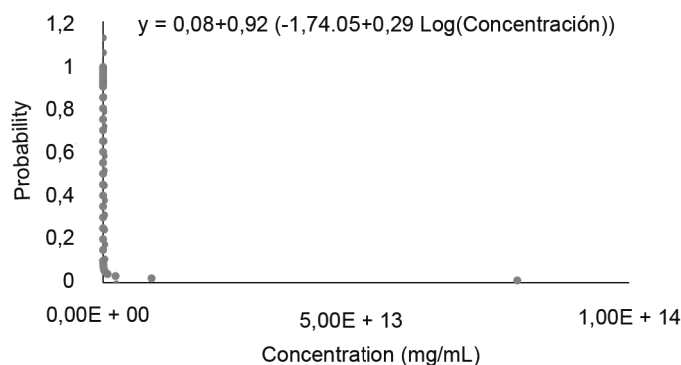


Fig. 1. Dose-response curve of the oil from the *J. curcas* fruit in engorged *R. (B.) microplus* females.

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