Scientific Paper

Repellent effect of the oil from *Jatropha curcas* L. seeds on larvae of *Rhipicephalus* (*Boophilus*) microplus (Canestrini, 1887) (Acari: Ixodidae)

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Abstract

Objective: To evaluate the repellent activity of the oil from *Jatropha curcas* L. seeds with different storage periods on larvae of *Rhipicephalus (Boophilus) microplus.*

Materials and Methods: The research was conducted in the parasitology and biotechnology laboratories of the Pastures and Forages Research Station Indio Hatuey, located in the Perico municipality, Matanzas province, Cuba. The tick strain Cayo Coco was used and oils with two storage periods were evaluated: one and three years of extraction. As positive control the acaricide Deltametrina (Butox®) was used and as negative one, distilled water. For the *in vitro* results the methodology for the identification of repellent substances, described by Chagas and Dias (2012), was used, where the concentrations of 0,5; 1,75; 2,5; 5 and 10 mg mL^{-1}, were used.

Results: The analysis showed significant differences ($p < 0,05$) between the experimental treatments in the different times, with regards to the negative control (distilled water). The repellence percentages were higher than 90 % for the *J. curcas* oils. The repellent activity was high, and the interval of 4-8 hours was the one with the best results, with values of 93,9 and 97,3 % for the 2014 and 2017 oil, respectively, without significant differences between both. After 8 hours, the positive control decreased its repellent activity until reaching values of 64,2 % at 16 hours. The distilled water did not show repellent activity, because its values were in an interval between 3 and 7 % during the experimental stage.

Conclusions: The *J. curcas* oil has repellent activity, independently from the storage time, with values that exceed 90 % of efficacy.

Keywords: larvae, *Rhipicephalus* (Boophilus) *microplus*, animal health

Introduction

Rhipicephalus (Boophilus) microplus (Canestrini, 1887) (Acari: Ixodidae) is considered the ectoparasite of higher importance for cattle husbandry. It has wide geographical distribution and is found, mainly, in the tropics and subtropics. It affects up to 80 % of the cattle population in the world, due to its capacity to adapt to the most varied ecological conditions (Aguilar-Tipacamú *et al.,* 2016; Ali *et al.,* 2016).

The presence of high infestations of the tick *R. (B.) microplus* in cattle can cause changes in the balance of the epidemiology of pathogens and in their respective diseases. An example of this is constituted by the clinical manifestations known as cattle tick fever (Santos *et al.,* 2019).

Tick resistance to antiparasitic drugs is one of the largest problems faced by the animal husbandry sector, because the availability of these products is increasingly scarce. The intensive use of these chemical products has provoked soil and water contamination, exposure of rural workers and presence of chemical residues in the milk and meat, which causes concern in the society and governmental agencies (Torres-Acosta *et al.,* 2015).

The use of plant-derived products constitutes an alternative way to the synthetic products that are utilized for the control of insects and mites. This is due to the presence of secondary metabolites, produced as a defense mechanism against the biotic and abiotic stress (Arceo-Medina *et al.,* 2016; 2017).

Bioactive compounds of botanical origin have advantages as an alternative to chemical synthesis products, because they have low toxicity, are soluble in water and are better degraded in the environment, due to the effect of solar radiation and humidity. For their preparation different plant parts (stems, leaves, root, bark, fruits, flowers and seeds) are used. The oils present in the plant species are widely used due to their therapeutic, bactericidal, fungicidal and insecticidal activity. Their efficacy can vary depending on the season, concentration of metabolites and environmental conditions under

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which the plants grow (Adenubi *et al.,* 2016; Barros *et al.,* 2019).

They include those from seeds of *Jatropha curcas* L., which is a multipurpose species, of tropical origin, belonging to the family *Euphorbiaceae* (Toral-Pérez *et al.,* 2016). *J. curcas* is considered an oil plant due to the oil content its seeds have. However, it is not commercialized because of the toxic effects that are ascribed to the presence of phorbol esters and a toxic protein called curcin (Lopera-Vélez *et al*., 2017).

The utilization of *J. curcas* in traditional medicine and its veterinary use have been documented in Asia, Africa and Latin America. All the parts of the plant have medicinal uses and are widely used in disease control, due to its properties as insecticide and fungicide (Valdés-Izaguirre *et al*., 2018). However, the highest number of studies about this plant is linked to human health and, to a lower extent, to its uses in veterinary medicine. Hence the importance of continuing the research about the potentialities of this species for animal health under tropical conditions.

The objective of the work was to evaluate the repellent activity of the oil from *J. curcas* seeds, with different storage periods on larvae of *R. (B.) microplus*.

Materials and Methods

Location. The research was conducted ate the parasitology and biotechnology laboratories of the Pastures and Forages Research Station Indio Hatuey (EEPFIH, for its initials in Spanish), which is located at 20º 50´ North latitude and 79° 32´ West longitude, in the Perico municipality, Matanzas province, Cuba, at an altitude of 19 m.a.s.l. Larvae of the Cayo Coco strain of *R. (B.) microplus*, were used, obtained by the National Laboratory of Veterinary Parasitology, belonging to the San Antonio de los Baños municipality, Artemisa province, Cuba.

Provenance, obtainment and characterization of the J. curcas oil. For extracting the oil, fruits of *J. curcas*, Cape Verde provenance, were collected in the Paraguay Farm, Guantánamo, Cuba. The mature fruits were dried under sunlight and the bark was eliminated to obtain the seeds, according to the methodology described by Sotolongo-Pérez *et al.* (2007). Oils with two storage periods were used: three years (extraction in 2014) and one year (extraction in 2017). The oils were preserved in airtight plastic containers, at ambient temperature and in a dark place until the beginning of the studies.

Treatment and experimental design. For the evaluation of the repellent activity of the seed oil from the *J. curcas* fruit, a complete randomized design was used, with four treatments, three replicas per treatment and 100 larvae per each replica. The essay lasted 8 h. The evaluated treatments were: T1) negative control (distilled water); T2) *J. curcas* oil, extraction in 2014, storage period of three years; T3) *J. curcas* oil, extraction in 2017, storage period of one year, and a positive control Deltametrina (Butox®) in a dose of 10 mg mL^{-1}. The oil concentrations were: 0,5; 1,75; 2,5; 5 and 10 mg mL^{-1}.

Experimental procedure. For obtaining the larvae donor animals, artificially infested and placed under isolation conditions, on timber boards, were used. Since 28 days, the engorged ticks were collected, once they detached on their own. They were transferred to the laboratory, washed with 1 % chlorinated distilled water and were dried with paper towels. Afterwards, they were placed in the incubator to do the egg laying, hatching and obtainment of the larvae. Larvae of 21 days of age were used and for the selection the characteristics described by Farias *et al.* (2012) were taken into consideration: presence of a whole body, normal appearance and motility. For this experiment the test of detection of substances with repellent activity on larvae, described by Chagas and Rabelo (2012), was utilized.

Wooden sticks of 25 cm of length were used, previously slotted at 5 cm, to determine the measurement area. The sticks were imbibed during 15 min in the top 10 cm of each evaluated substance, which was stored in Erlenmeyer flask. After the immersion, each wooden stick was inserted in 50-mL plastic vases, previously melted with paraffin. Then, a circle of quantitative filter paper, of 12 cm diameter, was fixed on the vase basis. The set was placed on Petri dishes with 5 mL of water at the bottom to prevent the larvae from escaping. With the aid of an inoculation loop, approximately 100 larvae were put on the filter paper (Chagas and Rabelo, 2012).

Because of the volatilization of bioactive compounds, the treatments were separated by a minimum distance of 2 m. Meanwhile, the negative and positive controls were placed in other rooms, to prevent interference of the results, at constant temperature and humidity of 21 ºC and 70 %, respectively. In addition, the access of people to the laboratory was limited to avoid the mixture of external smells with those of the evaluated substances.

Experimental measurements. The measurements were performed in the Biotechnology Laboratory of the EEPFIH. The organoleptic characteristics smell and color were considered. Likewise, the physical chemical properties of the oils (table 1) were evaluated: pH, density (kg m^{-3}), humidity (%), acidity index (%) and viscosity (mm^2s^{-1}). For the determination of the physical-chemical properties of the oil the ASTM/AOCS Rules were considered, according to the recommendation made by Lafargue-Pérez *et al.* (2012).

Repellent activity of the oils. The repellent activity was evaluated at 2, 4, 8, 16 and 32 h. The larval count was done through double adhesive tape, collecting the larvae independently for each area. The reading was performed as follows:

- Area 1. Larvae that were found in the first 5 cm of the end of the sticks impregnated with the substance.
- Area 2. Larvae that were found in the next 5 cm (between 5 and 10 cm) of the end of the sticks.
- Area 3. Larvae that were found in the not impregnated 15 cm of the sticks, on the filter paper and the paraffin.

The distribution percentage of the larvae for each one of the areas was determined. From the quantity of larvae in area 3, the repellence percentage in each treatment was calculated, according to the formula:

% of repellence = [(larvae of area 3/total larvae) x 100].

Statistical analysis. For the analysis of percentage of repellence and of larvae distribution, as they did not fulfill the assumptions of variance analysis, the non-parametric Kruskal-Wallis test was used, with significance level of 5 %. In the statistical analysis the package IBM® SPSS® Statistics version 22 was used.

Results and Discussion

Repellent activity of the J. curcas oil against R. (B.) microplus larvae. Figure 1 shows the repellence percentages for the evaluated substances at 2, 4, 8, 16 and 32 h, respectively. The analysis showed significant differences ($p < 0,05$) between the experimental treatments with regards to the negative control (distilled water).

The repellent activity in the oils was high and the 4-8 h interval was the one with the best results, with values of 93,96 and 97,3 % for the 2014 and 2017 oil, respectively, without significant differences between them.

According to Chagas and Dias (2012), the works that approach the repellent effects against *R. (B.) microplus* larvae are not very common, because most of the *in vitro* tests aim at detecting the acaricide action of the product on the different phases of the biological cycle of ticks (larvae and engorged ticks). However, the repellent action can be an interesting response, which complements the lethal effect of some commercial tick killing agents available in the market. Hence the importance of this study.

After 8 h, the positive control (Butox®) decreased its repellent activity until reaching values of 66,5 % at 32 h and significantly differed ($p < 0,05$) from the experimental treatments (*J. curcas* oils), which maintained a similar activity to the initial one throughout the experiment. This could be related to the volatilization of the active principles present in the oil, because its highest effects are not due to the repellent activity present in the oil, but because of direct contact and penetration in the parasites, which causes affectations in the nervous, muscular, reproductive and digestive systems (Roque-López, 2015).

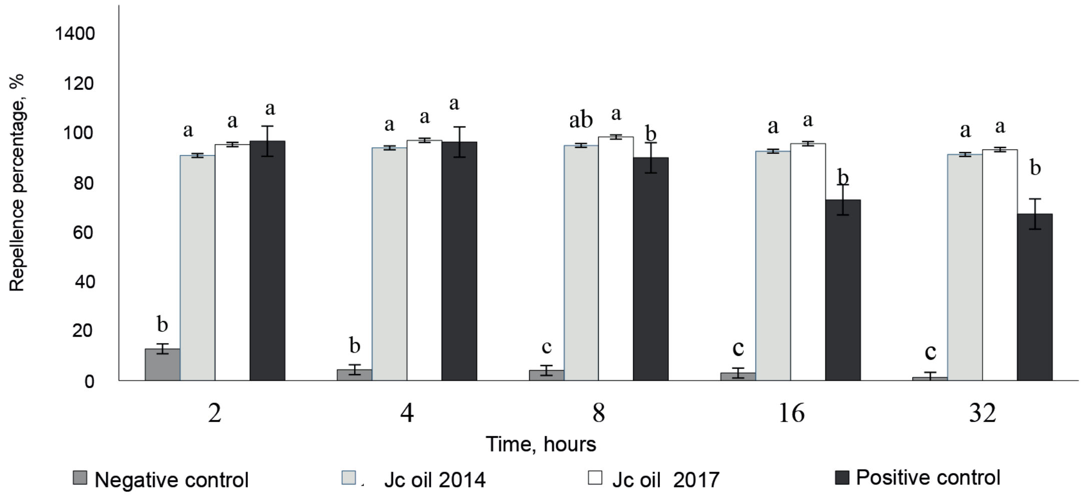
The distilled water did not show repellent activity, as was expected, because its values remained in an interval between 3 and 7 % during the experimental stage.

Table 2 shows the distribution of the percentage of *R. (B.) microplus* larvae for each evaluation moment (2, 4, 8, 16 and 32 h, respectively).

In general, it was observed that the larvae totally ascended to area 1 (0-5 cm), as in the case of the negative control (without repellent activity). An inverse process also occurred (area 3), when it corresponded to the *J. curcas* oils and the chemical control. Meanwhile, the quantity of larvae found in area 2 was variable and responded to the migratory process itself (negative geotropism) done by larvae in their biological cycle, characteristic that allows to define the repellent activity of substances.

Table 1. Characterization of the physical-chemical properties of the oil.

Oils	Density, kg m^{-3}	Humidity, %	Viscosity, mm^2s^{-1}	Acidity index, %
2014	910	0,05	31,5	95
2017	910	0,05	31,9	95



Different letters indicate significant differences between among treatments (p ≤ 0,05)

Figure 1. Repellent activity of the evaluated substances against *R.* (*B.*) *microplus* larvae.

Table 2. Distribution of the percentage of *R. (B.) microplus* larvae.

Time	Sampled area	Treatment				P - value	SE ±
		Negative control	Oil 2014	Oil 2017	Positive control		
	1	69,9^{b}	1,3^{b}	1,7^{b}	0,00^{e}		
2 h	2	17,4^{c}	8,7^{d}	4,0^{d}	4,4^{d}	0,0230	11,27
	3	12,7^{c}	89,9^{a}	94,3^{a}	95,6^{a}		
	1	70,8^{b}	2,7^{c}	1,3^{c}	1,3^{c}		
4 h	2	24,8^{b}	4,4^{c}	2,7^{c}	4,7^{c}	0,0545	11,52
	3	4,4^{c}	93,0^{a}	96,0^{a}	94,0^{a}		
	1	93,6^{a}	1,7^{b}	0,3^{c}	2,3 ^{b}		
8 h	2	2,4^{b}	4,7^{b}	2,4^{b}	8,7^{b}	0,0153	12,29
	3	4,0^{c}	93,7^{a}	97,3^{a}	89,0^{a}		
	1	93,3^{a}	1,3^{d}	2,7^{d}	10,4 ^{c}		
16 h	2	3,7^{d}	7,0^{d}	3,0^{d}	17,4 ^{c}	0,218	11,31
	3	3,0^{d}	91,6^{a}	94,3^{a}	72,2^{b}		
	1	94,6^{a}	3,7^{d}	2,0^{d}	10,0^{c}		
32 h	2	4,0^{d}	5,7^{d}	5,7^{d}	23,4^{c}	0,230	11,05
	3	1,3^{d}	90,6^{a}	92,3^{a}	66,6^{b}		

a, b, c and d: Different letters in the same column indicate significant differences among treatments (p ≤ 0,05)

The negative control showed the lowest values of larvae in area 3: 12,7; 4,4; 4,0; 3,0 and 1,3 % at 2, 4, 8, 16 and 32 h, respectively. This confirms that distilled water is a low-repellence substance.

At 2 and 4 h (table 1), the quantity of larvae recovered in the negative control showed significant differences (p < 0,05) among the areas, with the highest percentage for area 1 (69,9 and 70,8 %, respectively). Yet, in the experimental treatments (Jc oil 2014 and 2017) and in the chemical product, no differences were found between areas 1 and 2, but they were found with regards to area 3.

According to Jaenson *et al.* (2005), a repellent substance makes the organism perform movements in the opposed direction to the stimulation source. This behavior could be seen at 8, 16 and 32 h, moments at which the larvae migrated towards area 3, with the exception of the negative control.

Under natural conditions, the larvae migrate towards the edges of leaves and seek the meeting with the host. This behavior was observed in the negative control (distilled water), which proves that this substance does not have repellent activity, because the larvae in more than 94 % were grouped in area 1. According to García *et al.* (2019), this grouping characteristic of the larvae is a mechanism that allows them to survive under ambient conditions.

Furlong *et al.* (2002a, 2002b) stated that the larvae tend to migrate vertically, just below the end of the pasture leaves to escape adverse conditions, thus they avoid the energy expense and desiccation. Because of this behavior, it can be inferred that these substances have potential to limit the biological activity of larvae under natural conditions.

The experimental treatments did not show significant differences. The oil that was extracted in 2014, after a storage time of three years, maintains the repellence and efficiency properties.

According to Joshi *et al.* (2013), the oil quality and the concentration of its active principles is directly related to the storage conditions and the loss of some of its compounds by volatilization, because temperature and relative humidity are among the variables with higher influence on this performance.

The repellent effect of *J. curcas* can be determined by its content of fatty acids, such as palmitic, stearic and linoleic, and by the presence of secondary metabolites like alkaloids, steroids, tannins, flavonoids, phenol and coumarins (Rampadarath *et al.,* 2016), which provide it with a characteristic smell. These smells contribute to interrupt the search activity carried out by the larvae, and thus their biological cycle and the access to hosts under grazing conditions are hindered. Camacho and Peralta (2019) have reported repellent effects similar to the ones in this study, for the species origanum (*Origanum vulgare* L.) and rosemary (*Rosmarinus officinalis* L.).

Conclusions

The *J. curcas* oil shows repellent activity against *R. (B.) microplus* larvae, with effectiveness higher than 90 %. The repellent effect persists in time with higher results than the control in storage periods of three years.

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Conflict of interests

The authors declare that there is no conflict of interests among them.

Authors' contribution

- *Alberto Rizo-Borrego*. Design and setting up of the research, data analysis and interpretation, manuscript writing and revision.
- *Mildrey Soca-Pérez*. Design and advisory of the research, manuscript writing and revision.
- *Dany Eugenio García-Marrero*. Data analysis and interpretation.
- *Javier Arece-García*. Data analysis and interpretation.

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