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Effects of carvacrol on oocyte development in semi-engorged *Rhipicephalus sanguineus* sensu lato females ticks (Acari: Ixodidae)

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ABSTRACT

Currently, the most commonly used method to control ticks is the use of synthetic acaricides. However, these compounds are potentially harmful to hosts and the environment, in addition to causing the selection of resistant individuals. Therefore, several studies have been conducted to find sustainable methods to control ticks, such as *Rhipicephalus sanguineus* sensu lato, the most important vectors of pathogens for dogs. There has been increasing research on natural products with acaricidal action, especially with regard to plant-derived compounds as carvacrol, an aromatic monoterpene with several biological properties, including repellent and acaricidal activities, besides exerting cytotoxic effects on the exposed ticks. The objective of the present study was to evaluate the action of different carvacrol concentrations on the ovaries morphophysiology in semi-engorged *R. sanguineus* s.l. females to determine its effect on oocyte development. The results showed the occurrence of significant morphological alterations in the shape of oocytes (from round-shaped to irregular) and in the germinal vesicles, in addition to extensive cytoplasmic vacuolation.These effects were observed after the application of carvacrol at a concentration of 20 µL/mL. The most significant alterations were observed at the highest concentration (100 µL/mL), at which the oocytes could not develop further than stage II (total absence of oocytes III, IV and V). These data showed that even though carvacrol was unable to kill all ticks at these concentrations, surviving females could have had an altered reproduction, which would hinder the generation of new individuals, resulting in a long-term control. Data regarding the inhibition of oocyte development are unprecedented and indicate the use of carvacrol as a natural product with the potential to control *R. sanguineus* s.l. ticks.

1. Introduction

The search for new acaricide formulations to control *Rhipicephalus sanguineus* s.l. has been intensified, since it is an ectoparasite of significant environmental and sanitary importance, transmitting several biopathogens to animals and occasionally to human beings (Dantas-Torres, 2010; Paz et al., 2008). Different methods have been developed to control tick infestations, such as vaccines (Parizi et al., 2012), natural predators like the cattle egret (*Bubulcus ibis)* (Burtis et al., 2016), pheromone application (Benelli et al., 2016) and biological control with *Metahizium anisopliae* fungi (Webster et al., 2018).

Currently, the main control method for ticks is the use of synthetic acaricides. However, the prolonged or in inappropriate use of acaricides can cause the selection of resistant individuals and accumulation of chemical residues in the environment (Rodriguez-Vivas et al., 2018). In addition, synthetic acaricides are expensive and require specialized handling (Abbas et al., 2014). In this sense, the use of essential oils and their secondary metabolites (active principles), such as neem oil (Choudhury, 2009; Denardi et al., 2010; Remedio et al., 2015; Srivastava et al., 2008), andiroba oil (Farias et al., 2009; Roma et al., 2013; Vendramini et al., 2012) and castor oil esters (Arnosti et al., 2011; Sampieri et al., 2013), is an excellent strategy for the control of ticks, since such compounds are inexpensive, cause no environmental damage, and have low potential for fostering the development of resistant strains (Rosado-Aguilar et al., 2010).

Carvacrol, the object of study here, is a volatile phenolic monoterpene, and the active principle found in essential oils of plants in the families Lamiaceae and Verbenaceae, which include the genera

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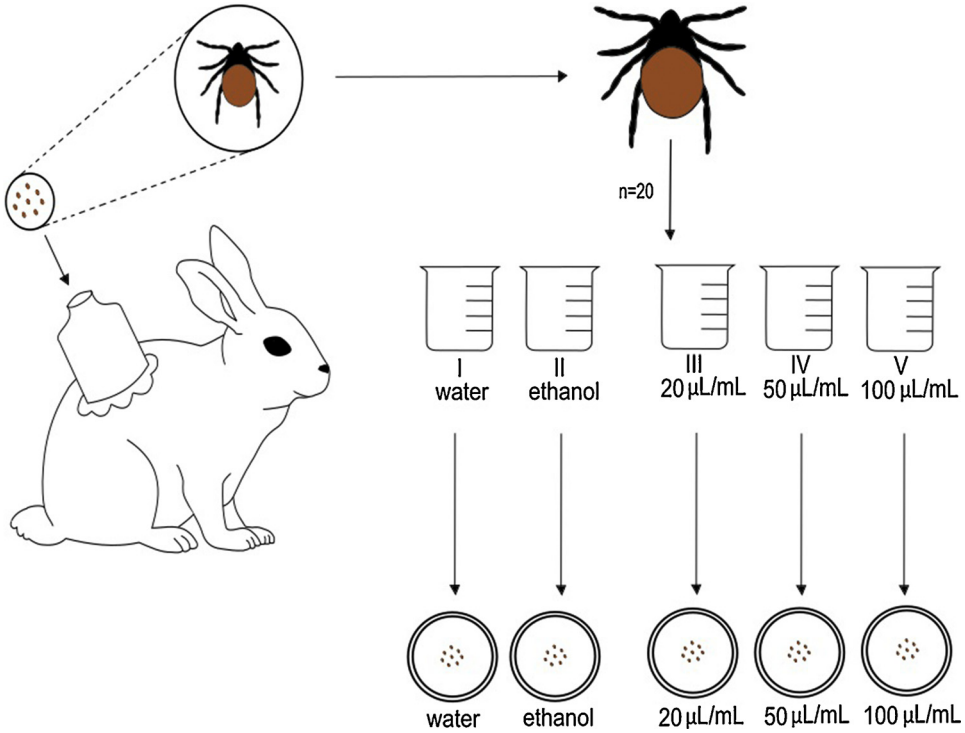

Fig. 1. Schematic drawing showing the steps of the method for exposure of semi-engorged females of *R. sanguineus* s.l. to carvacrol at various concentrations.

Origanum and *Lippia,* respectively (Cacciatore et al., 2015; Koc et al., 2013; Martinez-Velazquez et al., 2011; de Oliveira-Cruz et al., 2013). Several studies have demonstrated their various biological properties, such as antimicrobial, insecticide and acaricide (Jayakumar et al., 2012; Miladi et al., 2016; Tunç et al., 2016). Carvacrol showed extensive acaricidal activity against *Amblyomma americanum*, *Hyalomma marginatum*, *Rhipicephalus turanicus* and *R. sanguineus* s.l. (Cetin et al., 2010; Jordan et al., 2011; Koc et al., 2013; Senra et al., 2013).

One way to evaluate the potential of a compound in tick control is to study its effects on the morphology of tick internal organs, especially the ovary (Arnosti et al., 2011; Denardi et al., 2012; Oliveira et al., 2009; Roma et al., 2011; Sampieri et al., 2013; Vendramini et al., 2012). Accordingly, the objective of the present study was to investigate the effects of carvacrol at concentrations of 20, 50 and 100 µL/mL on the morphophysiology of the ovaries of semi-engorged *R. sanguineus* s.l. ticks.

2. Material and methods

2.1. Carvacrol

Carvacrol (5-isopropyl-2-methylphenol, CAS 499-75-2, molecular formula $C_{10}H_{14}O$, 99% pure) was purchased from Sigma-Aldrich (Sao Paulo, SP, Brazil).

2.2. Rhipicephalus sanguineus s.l. ticks

Unfed females and males of *Rhipicephalus sanguineus* s. l. ticks were used in the bioassays. The ticks were obtained directly from the colony maintained in a Biological Oxygen Demand (BOD) incubator under controlled conditions (28 °C, 85% humidity, and 12-h photoperiod), in the Animal Facility of the Department of Biology – UNESP, Rio Claro campus/São Paulo, Brazil.

One hundred *R. sanguineus* s. l. couples were released in special feeding chambers attached with an non-toxic glue (Unna paste, composed by 100 mL of glycerin (C_3H_8O3), 160 mL distilled water, 80 g agar-agar and 60 g zinc oxide) to the back of rabbits (Grupo Genético de Botucatu) weighing 3–3.5 kg and without previous exposure to ticks or acaricides. The rabbits, provided by the Genetic Group of Botucatu Campus, SP, Brazil, were kept in cages and received water and commercial food *ad libitum*. After feeding for four days, the semi-engorged female (mean weight ± SD = 26 ± 3.5 mg, p > 005) ticks were collected and subjected to the bioassays. All experimental procedures were performed according to Bechara et al. (1995) and were approved by the Ethics Committee in Animal Use (CEUA, UNESP Rio Claro/SP, Brazil), protocol No. 3822, 012/2015.

2.3. Experimental details

2.3.1. Bioassays (carvacrol)

The bioassays performed here were based on the highest carvacrol concentration used by Senra et al. (2013), who studied the effects of this compound on *R. sanguineus* s.l. larvae and nymphs. Using these data, five experimental groups were established, consisting of twenty individuals each.

Three treatment groups (T1-3) were exposed to 20, 50 and 100 µL/mL carvacrol, and two control groups were exposed to distilled water (C1) and solvent (50% ethanol) (C2). Ethanol was used as the solvent, because Chagas et al. (2003) found that up to 75%, it was not lethal to *R.* (*Boophilus*) *microplus* ticks, as were some other solvents.

Twenty *R. sanguineus* s.l. females were immersed in each solution for 5 min, according to the protocol established by Drummond et al. (1973). The same procedure was performed with the individuals from the control groups (distilled water and 50% ethanol). After immersion, the ticks were dried on absorbent paper, placed in labeled Petri dishes and covered with perforated plastic film for aeration (Fig. 1).

The samples were kept in an Eletrolab El 202 BOD incubator at 27 ± 1 °C and relative humidity of 80 ± 10% for 7 days. Control ticks were kept in a separate incubator so as to reduce the likelihood of cross-contamination with (the volatile) carvacrol.

2.3.2. Histology

Following thermal shock (females were kept in freezer at 2 °C for 1 min, this process lowers their metabolism without causing death), the