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Assessment and determination of LC_{50} of carvacrol and salicylic acid analogues with acaricide activity in larvae and adult ticks of *Rhipicephalus* (*Boophilus*) *microplus*

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A B S T R A C T

Rhipicephalus (*Boophilus*) *microplus* is a tick that causes huge economic losses in cattle. The indiscriminate use of acaricides has generated resistance to most compounds present on the market. Carvacrol and salicylic acid have been widely studied for their biological activities and have been evaluated in different strains of *Rhipicephalus*. In this research the analogues carvacrol and salicylic acid were evaluated in larvae of *R.* (*B.*) *microplus* with data obtained in larval packet test (LPT) and larval immersion test (LIT). A lethal concentration 50 (LC_{50}) was assessed. The most potent compounds were evaluated in the adult ticks since there are no reports of evaluation in the life state of the parasite. From all the tested compounds, the ethyl 2-methoxybenzoate (91.82 ± 1.66%, 0.91 μmol/mL) and ethyl 2,5-dihydroxybenzoate (89.14 ± 1.61%, 2.04 μmol/mL) showed the highest percentage of mortality and the lowest LC_{50}. They were found to be the best candidates for a study in vivo.

1. Introduction

R. (*B.*) *microplus* is one of the main ectoparasites of cattle in the tropical and subtropical regions in the world (Borges et al., 2011). The export of cattle to the United States is an economically important activity for Mexican livestock since it generates foreign exchange for about $ 700 million USD annually (González and Hernández, 2012). In Mexico, this tick causes huge economic losses in the livestock sector of approximately 48 million dollars because of direct damage such as damage to the skin by bites, blood loss, as well as indirect damage (transmission of the etiological agent), thus decreasing production and causing cost parameters for the control of the parasites such as: *Anaplasma marginale*, *Anaplasma centrale*, *Babesia bigemina*, *Babesia bovis*, *Borrelia theileri.* (Alonso et al., 2006; Rosario et al., 2011).

Since the indiscriminate use of acaricides has caused *R.* (*B.*) *microplus* to develop resistance to most acaricides available, it is necessary to find new compounds with acaricide activity. Carvacrol (Du et al., 2008; Di Pasqua et al., 2007; Cristani et al., 2007; Yin et al., 2012; Jayakumar et al., 2012; Ultee and Smid, 2001) and salicylic acid (Rangel et al., 2010; Mackowiak, 2000) have been studied extensively for their biological activities and have been evaluated in different strains of *Rhipicephalus*

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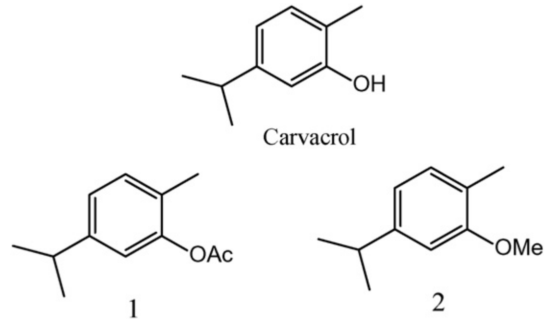

Fig. 1. Analogues of Carvacrol: carvacrol acetate (**1**) and carvacrol methyl ether (**2**).

(Coskun et al., 2008; Silveira et al., 2007). In this study the acaricidal activities of carvacrol analogues (carvacrol acetate (1) and carvacrol methyl ether (2) (Fig. 1) and salicylic acid analogues (2,5-dihydroxybenzoic acid (3), methyl 2,5-dihydroxybenzoate (4), ethyl 2,5-dihydroxybenzoate (5), 2-(α, α'-dimethoxymethyl)phenol (6), ethyl 2-methoxybenzoate (7), 2-hydroxybenzyl alcohol (8), methyl *p*-hydroxybenzoate (9) and propyl *p*-hydroxybenzoate (10) (Fig. 2), were measured by calculating the larval mortality percentage in a strain of *R.* (*B.*) *microplus*. The most potent compounds were selected according to the lethal concentration 50 (LC_{50}) and selected compounds were tested on the adult tick of this strain.

2. Materials and methods

Carvacrol, salicylic acid, 2,5-dihydroxybenzoic acid (**3**), methyl *p*-hydroxybenzoate (**9**) and propyl *p*-hydroxybenzoate (**10**) were purchased commercially. Carvacrol and salicylic acid analogues (carvacrol acetate (**1**), carvacrol methyl ether (**2**), methyl 2,5-dihydroxybenzoate (**4**), ethyl 2,5-dihydroxybenzoate (**5**), 2-(α,α'-dimethoxymethyl) phenol (**6**), ethyl 2-methoxybenzoate (**7**)) were prepared as described in the literature (Briard et al., 2008; Pilyugin et al., 2004; Yan et al., 2010; Liable et al., 2001; Ben Arfa et al., 2006). The 2-hydroxybenzyl alcohol (**8**) was provided by Dr. Héctor J. Salgado Z. obtained by a *Novo* technique.

2.1. Obtaining larvae of Rhipicephalus (B.) microplus

Adult ticks from cattle previously infected with a susceptible strain of *R.* (*B.*) *microplus* were donated by the Centro Nacional de Servicios de Constatación en Salud Animal (CENAPA) located in Jiutepec, Morelos, México; They were incubated during a two-week period at 28 °C and 80% relative humidity; the eggs were then collected in vials and incubated under the same conditions. After 14 days the hatched larvae were suitable for the bioassay (Bravo et al., 2008).

2.2. Acaricidal activity evaluation in vitro larvae R. (B.) microplus

There are two techniques for in vitro evaluation of the larval stage: LTP (Larval Packet Test; lipo-soluble compounds), LIT (larval immersion test; water-soluble compounds). The type of procedure depends on the solubility of the compound being evaluated.

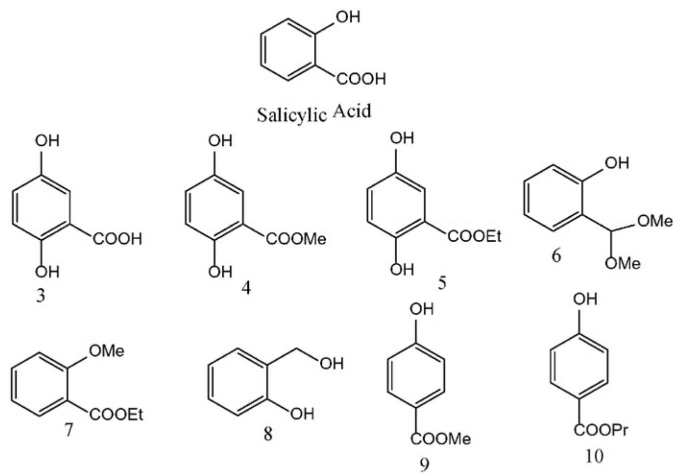

Fig. 2. Analogues of Salicylic acid: 2,5-dihydroxybenzoic acid (**3**), methyl 2,5-dihydroxybenzoate (**4**), ethyl 2,5-dihydroxybenzoate (**5**), 2-(α,α'-dimethoxymethyl)phenol (**6**), ethyl 2-methoxybenzoate (**7**), alcohol 2-hydroxybenzyl (**8**), methyl *p*-hydroxybenzoate (**9**) and propyl *p*-hydroxybenzoate (**10**).