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In-vitro efficacy of a botanical acaricide and its active ingredients against larvae of susceptible and acaricide-resistant strains of *Rhipicephalus* (*Boophilus*) *microplus* Canestrini (Acari: Ixodidae)

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ABSTRACT

Ticks and tick-borne diseases are a major constraint for a sustainable cattle industry in the tropical and sub-tropical regions of the world. The development of resistance to most of the commonly used acaricides has led to an assessment of herbal products for acaricidal activity as an eco-friendly tick control alternative. A botanical product, Essentria® IC-3 insect concentrate containing rosemary oil (10%), geraniol (5%) and peppermint oil (2%), acts on target pests by blocking octopamine. Essentria® IC-3 and its active components were evaluated for larvicidal activity against several susceptible and acaricide-resistant strains of the cattle fever tick, *Rhipicephalus* (*Boophilus*) *microplus* Canestrini by Larval Packet test using 14–21 d old unfed larvae. The efficacy was assessed by measuring percent larval mortality and estimating lethal concentrations at 50% (LC_{50}) and 95% (LC_{95}) with 95% confidence limits (CL) using probit analysis. The LC_{50} and LC_{95} (95% CL) values for Essentria® IC-3 against the susceptible strain were estimated as 0.647% (0.59–0.69) and 1.033% (0.94–1.19), respectively, whereas, LC_{50} and LC_{95} values for other strains were variable, ranging from 0.597-1.674% and 0.927–2.236%, respectively. Among the various active ingredients, the larvicidal property of Essentria® IC-3 seem to be attributable mainly to geraniol and the LC_{50} and LC_{95} (95% CL) values for geraniol against the susceptible Deutch strain were estimated as 0.656% (0.61–0.69) and 1.114% (1.03–1.25), respectively. The comparison of LC_{50} and LC_{95} values of acaricide-resistant strains showed susceptibility comparable to Deutch against geraniol except for the Las Palmas strain. We report a low level of resistance in some of the acaricide-resistant strains against the herbal acaricide in the cattle tick for the first time, possibly due to cross-resistance to chemical acaricides.

1. Introduction

Rhipicephalus (*Boophilus*) *microplus* Canestrini (Acari: Ixodidae) commonly known as “the southern cattle fever tick” or “southern cattle tick” in Texas is the most important tick of veterinary importance with a high economic impact on cattle husbandry throughout tropical and subtropical regions. Heavy tick infestations cause huge economic losses through anorexia, toxicosis, blood loss, general stress and irritation, decrease in productivity, depression of immune function, damage to hides, transmission of pathogens and treatment costs (Ghosh et al., 2007). Economic losses to cattle producers from ticks and tick-borne diseases are huge: Brazil and Australia report annual losses of USD 3.24 billion (Grisi et al., 2014) and AUD 175 million (Playford et al., 2005), respectively.

The currently available tools for tick control consist of chemical acaricides used with different application methods and various formulations, breeding of tick resistant cattle, anti-tick vaccines, biological control by pathogens or predators, pheromone-assisted control and botanical acaricides (reviewed by Benelli et al., 2016). However; large scale and repeated applications had limited their efficacy in reducing tick infestations and are often accompanied by serious drawbacks, including the development of acaricide resistant ticks, environmental contamination, and even contamination of milk and meat products with insecticide residues (Graf et al., 2004). These inherent disadvantages of

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chemical acaricides, high cost of developing new drugs and paucity of satisfactory immunizing agents have led to renewed interest in the use of botanicals for control of cattle ticks (Zaman et al., 2012). Botanical acaricides can be a suitable alternative for synthetic acaricides because of their low toxicity to non-target organisms including humans and the ability of rapid biodegradation of their residues.

The botanical product, Essentria® IC-3, according to the label, kills crawling and flying insect pests and can be used for fogging of animal quarters (cattle barns, horse barns, poultry barns, swine houses, zoos), dooryard turf and ornamentals, animals and mosquito misting applications. The active ingredients of this product are rosemary oil (10%), geraniol (5%) and peppermint oil (2%). Essentria® IC-3acts on target pests by blocking octopamine. It may be diluted with water or oil and applied with conventional application equipment with most effective results achieved when used as part of a treatment protocol that includes physical, environmental and other chemical pest control measures. It can be used as a livestock spray to control flying insects, ticks and lice when diluted at 1–3 fluid ounces per gallon of mineral oil. However, in the absence of a specific claim to control *R.* (*B.*) *microplus* the current study was executed to evaluate the acaricidal effect of this herbal formulation and its active ingredients, against various susceptible and acaricide-resistant strains of *R.* (*B.*) *microplus*.

2. Materials and methods

2.1. Acaricides

Essentria® IC-3 insect concentrate (Lot No. 57480), rosemary oil, geraniol and peppermint oil (Sigma-Aldrich, St. Louis, MO, USA) were used for the bioassays. Also, all active ingredients of Essentria® IC-3 were mixed as per label information (rosemary oil-10%, geraniol-5% and peppermint oil-2%) and used for bioassay to record the synergistic effect, if any.

2.2. Ticks

All tick populations used in this study were maintained at the Cattle Fever Tick Research Laboratory (CFTRL), Edinburg, Texas. *R.* (*B.*) *microplus* ticks rearing conditions at the CFTRL were described in Davey et al. (1980). We adhered to protocols for the care and use of animals as promulgated by the presiding Institutional Animal Care and Use Committee (IACUC). The facilities are fully accredited by the American Association of Laboratory Animal Care.

The following cattle tick colonies established in the CFTRL, Edinburg, Texas were used: (1.) Deutch strain (F62), as a susceptible reference in the bioassays; (2.) El Zamora (F32), originated in the state of Tamaulipas, Northeast Mexico, is resistant to synthetic pyrethroids (SP), organophosphates (OP), amitraz and fipronil (Miller et al., 2013). (3.) Yucatan (F19), originated in the state of Yucatan, Mexico, is resistant to SP, OP, amitraz and ivermectin (Rodríguez-Vivas et al., 2014). (4.) Santa Luiza (F58), originated in the state of Rio Grande do Sul, is resistant to SP and amitraz (Li et al., 2008). (5.) San Roman (F79), originated in the state of Yucatan, Mexico, is resistant to SP and OP; (6.) San Alfonso (F58), originated in the state of Guerrero, Mexico, is resistant to SP and amitraz; (7.) Las Palmas (F37), obtained from a cattle tick outbreak in Zapata Co. Texas, is susceptible to all acaricides; (8.) Lajas (F7), originated in Lajas, Puerto Rico, is susceptible to all acaricides; (9.) Gurwitz strain (F1), obtained from a cattle tick outbreak in Jim Wells Co., Texas, is susceptible to all acaricides; (10.) Sal Si Puedes (F1), obtained from an outbreak in Starr Co., Texas, is resistant to SP (data not shown).

2.3. Bioassays

After collection, engorged female ticks were placed in 5-cm diameter plastic Petri dishes, and held in an environmental chamber at 28 °C, 91% relative humidity (RH), and a photoperiod of 12:12 (L:D) h. After 20 d, eggs were collected, mixed thoroughly, weighed, and returned to the environmental chamber. Fourteen days after the first observation of larvae, a Food and Agriculture Organization (FAO) Larval Packet Test (LPT) (Stone and Haydock, 1962) was performed with Essentria® IC-3 and its active ingredients individually. The FAO of the United Nations (FAO, 1971) has described the LPT technique in details. Essentria® IC-3 and each of its active ingredients were diluted in two parts of trichloroethylene (TChE) (Sigma–Aldrich) and one part of olive oil (OO) (Sigma–Aldrich, Fluka). Essentria® IC-3 and its active ingredients were subsequently diluted in TChE:OO for obtaining concentrations of 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 and 3.0%. Each serial dilution had a negative control (diluent only) and each dose had three replicates. A volume of 0.7 ml of each dilution was applied to 8 × 9 cm filter paper (Whatman No. 1, Whatman, Madstone, United Kingdom) and TChE was allowed to evaporate under a fume hood for 2 h. After drying, the bioassay sheets were folded in half, and metal clips (Bulldog, Boston Clip No. 2, Hunt Manufacturing Co., Statesville, NC, USA) were placed on the sides, forming a packet. Approximately 100 larvae were placed into each packet, and the top was sealed with a third clip. Packets containing larvae were held at 28 °C, 70–80% RH, and photoperiod of 12:12 (L:D) h for 24 h. After 24 h, the packets were removed from the environmental chamber and opened, and the numbers of live and dead larvae were counted. Larvae that moved their legs but did not walk were counted as if dead. Additional tests with higher or lower doses were performed to obtain mortality ranging from 0 to 100%.

2.4. Statistical analysis

Probit analysis was conducted on bioassay results data using PoloPlus (Le Ora Software, 2004). This analysis included probit

Table 1
In vitro acaricidal efficacy of Essentria® IC-3 against unfed larvae of various strains of *R.* (*B.*) *microplus*.

Strain	N	Slope	LC_{50}(%) (95%CL)	LC_{95}(%) (95%CL)	[a]RR_{50} (95%CL)	[b]RR_{95} (95%CL)
Deutch[c]	2048	8.11 ± 0.45	0.647 (0.59–0.69)	1.033 (0.94–1.19)	1.0	1.0
El Zamora	2314	13.09 ± 0.96	1.674 (1.57–1.77)	2.236 (2.05–2.66)	2.586 (2.48–2.69)	2.165 (2.02–2.32)
Gurwitz	2863	4.56 ± 0.18	0.882 (0.83–0.94)	2.023 (1.83–2.31)	1.362 (1.29–1.43)	1.959 (1.80–2.13)
Lajas	2358	8.62 ± 0.52	0.597 (0.54–0.65)	0.927 (0.84–1.10)	0.922 (0.88–0.97)	0.897 (0.83–0.96)
Las Palmas	2650	8.68 ± 0.66	0.964 (0.89–1.02)	1.491 (1.35–1.80)	1.488 (1.42–1.55)	1.443 (1.33–1.56)
Sal Sui Puedes	2959	9.53 ± 0.76	0.876 (0.82–0.92)	1.304 (1.19–1.56)	1.354 (1.29–1.41)	1.263 (1.17–1.36)
San Alfonso	2838	5.86 ± 0.29	0.865 (0.77–0.95)	1.651 (1.45–2.03)	1.336 (1.27–1.40)	1.599 (1.48–1.73)
San Roman	4196	9.14 ± 0.41	1.022 (0.96–1.08)	1.546 (1.41–1.79)	1.578 (1.52–1.64)	1.497 (1.40–1.59)
Santa Luiza	2549	7.81 ± 0.41	0.644 (0.59–0.69)	1.046 (0.95–1.19)	0.994 (0.95–1.04)	1.012 (0.94–1.09)
Yucatan	2284	6.37 ± 0.28	0.942 (0.88–1.03)	1.707 (1.54–1.95)	1.455 (1.39–1.52)	1.652 (1.53–1.79)

[a] RR_{50}: LC_{50} of tick strain/LC_{50} of susceptible strain.
[b] RR_{95}: LC_{95} of tick strain/LC_{95} of susceptible strain.
[c] Susceptible strain.