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Acaricidal activity of the essential oil from *Tetradenia riparia* (Lamiaceae) on the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari; Ixodidae)

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ABSTRACT

Tetradenia riparia (Lamiaceae) is a well-known herbal medicine with a variety of useful properties, including its acaricidal effect. This experiment was carried out to study the bioacaricidal activity of *T. riparia* essential oil (EO) against engorged females of *Rhipicephalus* (*Boophilus*) *microplus* (Acari; Ixodidae). For this purpose, nine serial concentrations (12.50%, 6.25%, 3.75%, 1.80%, 0.90%, 0.45%, 0.22%, 0.11%, and 0.056% w/v) of *T. riparia* were used for the adult immersion test (AIT). For the larval packet test (LPT), we used 14 serial concentrations (100.00%, 50.00%, 25.00%, 12.50%, 6.25%, 3.65%, 1.82%, 0.91%, 0.45%, 0.228%, 0.114%, 0.057%, 0.028%, and 0.014% w/v). The results for AIT showed 100.00% and 2.05% mortality, 19.00 and 90.20% for the total number of eggs, egg-laying inhibition of 0.00% and 90.20%, hatchability inhibition of 0.00% and 70.23%, and product effectiveness of 100.00% and 2.89%, respectively. The AIT indicated that the LC_{50} and $LC_{99.9}$, calculated using the Probit test, were for mortality (%) 0.534 g/mL (0.436–0.632) and 1.552 g/mL (1.183–1.92); for total number of eggs were 0.449 g/mL (0.339–0.558) and 1.76 g/mL (1.27–2.248); and for hatchability inhibition were 0.114 g/mL (0.0–0.31) and 2.462 g/mL (1.501–3.422), respectively. Larvae between 14 and 21 days old were fasted and placed in each envelope. Bioassays were performed at 27° ± 1 °C, RH ⩾ 80%. Larval mortality was observed 24 h after treatment and showed 10.60–100% mortality in the LPT bioassay. The LPT showed that the LC_{50} and $LC_{99.9}$ were 1.222 g/mL (0.655–1.788) and 11.382 g/mL (7.84–14.91), respectively. A positive correlation between *T. riparia* EO concentration and tick control, was observed by the strong acaricidal effects against *R.* (*B.*) *microplus*, and the mortality rate of ticks was dose-dependent. Our results showed that *T. riparia* is a promising candidate as an acaricide against resistant strains of *R.* (*B.*) *microplus*.

1. Introduction

In Brazil, the main tick species that undermines the productivity of cattle raising is *Rhipicephalus* (*Boophilus*) *microplus*. The damage caused by this tick to South American cattle exceeds two billion dollars a year (Grisi et al., 2002). Primarily by biting, it affects the production of meat and milk but the tick also injects toxins into the host and transmits infectious agents (Olivo et al., 2008). The control of cattle ticks is usually done using conventional chemicals including synthetic pyrethroids (SP), organophosphates (OP), and amitraz (Am). These cause the rapid development of resistance to the active principle. Further, their use has caused great concern in society and government, by harming the animals themselves and humans who consume the products from these animals (Chagas et al., 2003). Each time that ticks survive an application of insecticide, they transmit genetic information to later generations about how to survive that product (Furlong et al., 2004). The use of plant extracts in tick control has also been the focus of extensive research (Martinez-Velazquez et al., 2010). *Tetradenia riparia* (Hochstetter) Codd, a member of the family Lamiaceae, is a shrub common throughout Africa. In South Africa, it is one of the most popular herbs and medicinal plants (Van Puyvelde and De Kimpe, 1998). For decades, *T. riparia* has been the subject of research to isolate and identify the active compounds present in extracts from its leaves. Several studies have been conducted to evaluate the biological activities of *T. riparia*: as larvicide (Weaver et al., 1992); insecticide (Weaver et al., 1994); antimalarial (Campbell et al., 1997) and repellent effects on *Anopheles gambiae*

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(Omolo et al., 2004). To date, no studies reported in the literature on the acaricidal activity of this plant. Thus the present experiment was to evaluate the acaricidal activity of the essential oil from fresh leaves of *T. riparia* against the acari of *R.* (*B.*) *microplus*.

2. Materials and methods

2.1. Plant material

Plant material (leaves) of *T. riparia* was collected in a medicinal-plant garden at the Universidade Paranaense (Unipar), northwestern Paraná State, Brazil. A voucher specimen was authenticated and deposited at the herbarium of the University Educational Paranaense (HEUP), under number 2502. To obtain the essential oil of *T. riparia*, fresh leaves were collected between 06:30 and 08:00 a.m. during summer, between 21/12/2008 and 21/03/2009, and hydrodistilled for 3 h using a modified Clevenger-type apparatus. The distilled oils were collected and dried over anhydrous sodium sulfate and stored in a freezer (Gazim et al., 2010).

2.2. Ticks

About 300 engorged females of *R.* (*B.*) *microplus* were collected from naturally infested cattle on farms in Tapejara city, Paraná, which had suspended the use of acaricidal treatment for at least 45 days prior to the study. The females were used in the AIT or incubated at 27–28 °C and 70–80% relative humidity for 2 weeks until they laid eggs.

2.3. Bioassays (AIT and LPT)

For the acaricidal test (AIT), we made serial dilutions (12.50%, 6.25%, 3.75%, 1.80%, 0.90%, 0.45%, 0.22%, 0.11%, and 0.055% w/v) of essential oil of *T. riparia*. For the larval packet test (LPT) the concentrations used were 100.00%, 25.00%, 12.50%, 6.25%, 3.65%, 1.82%, 0.91%, 0.45%, 0.228%, 0.114%, 0.057%, 0.028%, and 0.014% w/v. In both tests, the essential oil was diluted in an aqueous solution containing 2.0% of an emulsifying agent (Tween 80 v/v), described by Farias et al. (2007). The AIT was described by Drummond et al. (1976), where groups of 30 engorged females were weighed and immersed for 5 min in the respective dilutions (10 mL) in a 50-mL beaker which was gently agitated (three times) at room temperature. The emulsifying solution (2% Tween) was used as the negative control (Farias et al., 2007; Rosado-Aguilar et al., 2010). Water with 0.125% Colosso Pulverização® (cypermethrin 0.25; chlorpyrifos 0.25 and citronellal 0.01 g/mL) was used as a positive control. Ticks were removed from the solutions, dried, and each group placed in a separate Petri dish. The Petri dishes were incubated at 27–28 °C and 70–80% relative humidity. After 14 days, the number of females laying eggs was recorded, and the eggs were collected, weighed, and observed. The eggs were placed in glass tubes, incubated at 27–28 °C and 70–80% relative humidity, and after 21 days the percentage of hatched eggs was calculated. The effectiveness of the product (PE) was calculated using the mathematical formulas below, described by Drummond et al. (1973). Each treatment contained three replicates:

$$\text{Reproduction estimated (RE)} = \frac{\text{egg weight} \times \%\ \text{hatchability} \times 20.000^{*}}{\text{weight of females}}$$

*Constant indicating the number of eggs present in 1 g of egg laying.

$$\text{Effectiveness of the product (PE)} = \frac{\text{RE (control group)} - \text{RE (treated group)}}{\text{RE (control group)}} \times 100$$

To implement the LPT test, larvae were obtained from engorged females of *R.* (*B.*) *microplus*, and allowed to rest unfed for 14–21 days following hatchability prior to their use. The larvae were exposed to test solutions in filter-paper envelopes (2 × 2 cm), containing micropores to allow better ventilation (Fernandes et al., 2008). The following treatments were used: (1) envelopes of dry filter paper; (2) envelopes of filter paper moistened with a negative control (solution of 2% Tween 80 in distilled water); (3) positive controls consisting of water with 0.125% Colosso Pulverização® (cypermethrin 0.25; chlorpyrifos 0.25 and citronellal 0.01 g/mL) and (4) envelopes moistened with 2 mL of each concentration of essential oil of *T. riparia* to be tested. After each envelope was treated and larval ticks inserted, the opening was folded over (~10 mm) and re-sealed with a metallic clip, with its identification mark (solution and concentration tested) on the outside. The packets were placed in the BOD incubator at a temperature of 27–28 °C and 85–95% relative humidity for 24 h. The envelopes were then opened and inspected, using a stereomicroscope, to record the number of live and dead larvae, and any toxicological effects observed. Each treatment contained three replicates:

$$\text{Corrected percent mortality} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

2.4. Statistical analyses

The experimental design was completely randomized. The data were processed and subjected to analysis of variance (ANOVA), and differences between means were determined by Tukey test at 5% significance level. Lethal concentrations (LC) to kill 50% and 99% of larvae and their respective 95% confidence intervals (CI) were calculated by probit analysis (software R version 11.2).

3. Results

The essential oil of *T. riparia* was analyzed by Gazim et al. (2010), and 31 compounds, comprising 97.17% of the oil, were identified. The predominant class in this oil was oxygenated sesquiterpenes: 14-hydroxy-9-epi-cariophyllene (18.03%), *cis*-muurolol-5-en-4-α-ol (11.73%), ledol (7.18%), and α-cadinol (4.90%), followed by monoterpene hydrocarbons: limonene (3.69%), and oxygenated: fenchone (12.87%).

The percentages obtained for in vitro efficacy of the oil of *T. riparia* against *R.* (*B.*) *microplus* are shown in Table 1. In the AIT, the efficacy of treatment against engorged females was assessed by measuring mortality of gravid females, total number of eggs, egg weight, percentage of hatchability, and product efficiency (Table 1). Table 2 shows the percent mortality of *R.* (*B.*) *microplus* larvae exposed to different concentrations of *T. riparia* essential oil through the larval packet test (LPT). In dilutions of 100%, 50%, and 25% the oil killed 100% of the larvae, indicating a maximum efficiency. In dilutions from 12.5% to 0.014%, the mortality rate of larvae was high, ranging from 97.6% to 10.60%, respectively, with no significant differences between them. However, all these results differed significantly from the negative control (8.00%). The LC_{50} and $LC_{99.9}$ (Table 3) calculated using the Probit test, were for% mortality 0.534 g/mL (0.436–0.632) and 1.552 g/mL (1.183–1.92), for total number of eggs 0.449 g/mL (0.339–0.558) and 1.76 g/mL (1.27–2.248), and the hatchability inhibition was 0.114 g/mL (0.0–0.31) and 2.462 g/mL (1.501–3.422), respectively. The larval mortality (LPT) was 1.222 g/mL (0.655–1.788) and 11.382 g/mL (7.84–14.91), respectively.