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 (100.0%, 50.00%, 25.00%, 12.50%, 6.25%, 3.75%, 1.80%, 0.91%, 0.45%, 0.22%, 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 1.532 g/mL (1.183–1.92); for total number of eggs 0.449 g/mL (0.339–0.558) and 1.76 g/mL (1.501–3.422), respectively. Larvae between 14 and 21 days old were fasted and placed in each envelope. Bioassays were performed at 27°C ± 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*.
(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 placed in glass tubes, incubated at 27–28 °C and 85–95% relative humidity for 24 h. The tubes 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 0.655–1.788, 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.
The analyses of the essential oil from T. riparia by GC–MS identified a mixture of sesquiterpenes (50.30%) and monoterpenes (19.52%). The essential oil showed intense activity against different stages of R. (B.) microplus, and the mortality rate of the ticks was dose-dependent. Many essential oils and their components, mainly monoterpenoids and sesquiterpenoids, show repellent, chemosterilant, antifeeding, and biocidal activities against the cattle tick R. (B.) microplus (Facey et al., 2005). However, the chemical constituents of essential oils from other plant species differ from those found in the essential oil of T. riparia. For example, the oil of T. riparia killed 100% of gravid female ticks at concentrations from 1.8% to 12.5%. Similarly, the studies of Martins (2006) with essential oil of Cymbopogon winterianus (citronella or Java grass), which is rich in monoterpenoids (41.15% citronellal), showed 100% mortality at concentrations from 12.5% to 50.0%. Chagas et al. (2002) investigated the action of monoterpene-rich essential oil from three species of Eucalyptus: Eucalyptus citriodora (94.9% citronellal), Eucalyptus globulus (9.93% α-pinene), and Eucalyptus staigeriana (24.78% α-limone), in five different concentrations, against gravid female ticks. The results showed that E. citriodora, E. globulus and E. staigeriana killed 100% of females at mean concentrations of 17.50%, 15% and 12.5%, respectively.

Regarding the inhibitory action of T. riparia essential oil on ovi-position, there was a 100% reduction of egg laying with oil concentrations from 1.80% to 12.5%. This effect was also evaluated by Olivo et al. (2008), who investigated the effect of the essential oil from C. nardus containing high levels of monoterpenes (50.0% citronellal), and observed 90% inhibition of oviposition at concentrations of 100%, 50%, 25% and 10%. Also, Ribeiro et al. (2010) found that essential oil from H. ringens, which has a high content of oxygenated monoterpenes (86.0% Pulegone), inhibited egg laying by 76.4% and 48.2% at concentrations of 5.0% and 2.5%, respectively. Another parameter evaluated was the influence of T. riparia essential oil on the percentage of hatching of larvae. The results showed 100% inhibition of hatching at concentrations ranging from 1.8% to 12.5%. Ferrarini et al. (2008) evaluated the effect of limonene (monoterpene hydrocarbons) in inhibiting egg hatching of R. (B.) microplus, at concentrations from 0.25% to 1.0%, and obtained 91% to 100% inhibition. Taken together, the results showed that oil has a significant potential to control the larvae of R. (B.) microplus. Ferraz et al. (2010) observed that for the essential oil of Piper amalago, which contained high levels of monoterpenes (20.52% limonene) and sesquiterpenes (11.18% zingiberene), the most active concentration with LC50 was 0.23%. Ribeiro et al. (2008) evaluated the larvicidal effect of Drimys brasiliensis essential oil, which contained predominantly sesquiterpenes (30.4% cycloclorenone), on the larvae of R. (B.) microplus. One hundred percent of the larvae were killed at concentrations of 2.5%, 1.25% and 0.625%. Chagas et al. (2002) also observed that the essential oil of E. staigeriana killed 100% of the larvae at a concentration of 10%, and E. globulus had the same effect at a concentration of...
20%. The use of a pesticide composed of a mixture of a pyrethroid (cypermethrin) and an organophosphate insecticide (chlorpyrifos) has contributed greatly to agricultural development, but poses risks to the ecosystem (Zhou et al., 2011). In contrast, Chungsamarnyart, N., Jiwajinda, S., Ratanakreetakul, C., 1991. Practical extraction of andiroba (andiroba) no controle de Boophilus microplus (Acari: Ixodidae). Revista Brasileira de Plantas Medicinais 8, 71–78.

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References


