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## Comparison of three Larval Immersion Tests in Syringe to evaluate acaricidal activity of chemical solutions

## Comparação entre três técnicas de Imersão de Larvas em Seringas para avaliar atividade acaricida de soluções químicas

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### Abstract

The aim of this study was to compare Larval Immersion Test in syringes, originally proposed by Souza et al. (2008), with two modified bioassays proposed by Silva et al. (2011) and Sindhu et al. (2012). *Rhipicephalus (Boophilus) microplus* larvae were exposed to nine dilutions of commercial acaricide. After 24 hours, the number of dead larvae was counted. The LC<sub>50</sub> for the three methods were 0.186%, 0.125% and 0.153%, respectively. Mortality of larvae exposed to chemical dilutions on Silva et al. (2011) were significantly higher than other methods at lower concentrations, probably in consequence of longer exposure time of larvae to solutions. However, modifications proposed by Sindhu et al. (2012) make technic execution more practical and safer.

**Key words:** Syringe test. *In vitro*. *Rhipicephalus (Boophilus) microplus*.

### Resumo

O objetivo deste trabalho foi comparar a técnica de imersão de larvas em seringas proposta originalmente por Souza et al. (2008), com duas modificações propostas por Silva et al. (2011) e Sindhu et al. (2012). Larvas de *Rhipicephalus (Boophilus) microplus* foram expostas a nove diluições de acaricida comercial. Após 24 horas, contabilizou-se o número de larvas mortas. A CL<sub>50</sub> nos três métodos foi de 0,186%, 0,125% e 0,153%, respectivamente. Houve maior mortalidade de larvas no método de Silva et al. (2011), nas menores concentrações, quando comparado aos demais métodos, provavelmente devido ao maior tempo de exposição das larvas ao composto químico. Entretanto, as modificações propostas por Sindhu et al. (2012) proporcionam maior praticidade e segurança na execução da técnica.

**Palavras-chave:** Teste em seringa. *In vitro*. *Rhipicephalus (Boophilus) microplus*.

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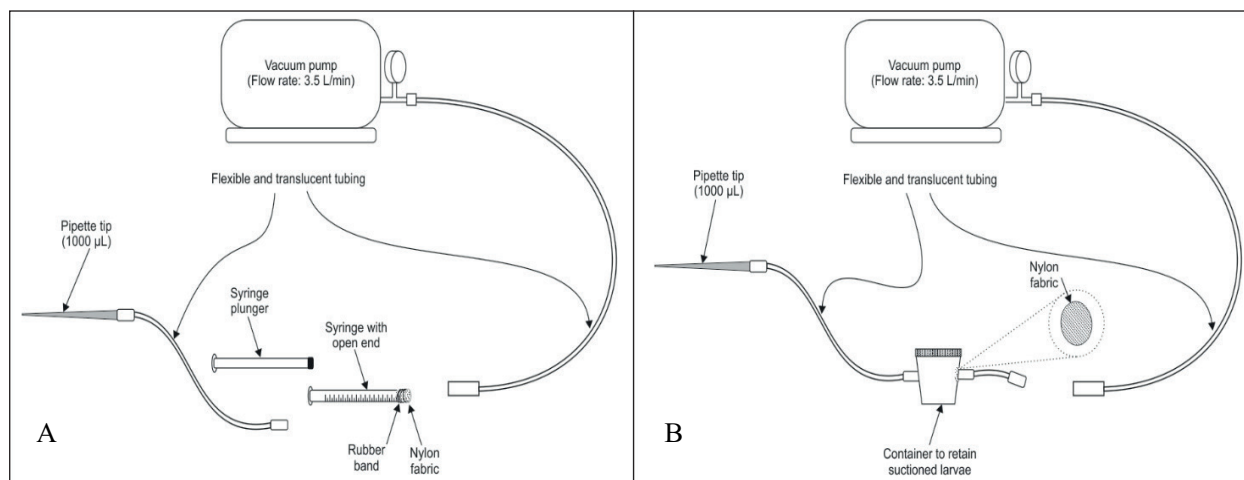
Chemical control of *Rhipicephalus (Boophilus) microplus* in Brazil is going through a critical moment of scarcity of still effective classes of drugs. The search for new active molecules has tempted studies on acaricidal effect of plant extracts. At present in vitro bioassays are currently used as screening tests. Souza et al. (2008) developed a method based on Larvae Immersion Test, first described by (SHAW, 1966). The method replaces the microtube by syringes making the original technic less laborious. The bioassay described by Souza et al. (2008) has been changed to evaluate plant extracts acaricide effect in Brazil and abroad (SILVA et al., 2011; SINDHU et al., 2012). This paper aimed to compare the proposed adaptations to the original method. Souza et al. (2008) method consists in immersing 5 mL syringe in test solution to submerge tick larvae for 30 seconds. Acaricidal effect is determined counting the number of live and dead larvae 24 hours after exposition. Syringes used in the bioassay are prepared cutting the nozzle and making an orifice of 1 mm of diameter in the middle of the syringe to permit scape of air and liquid entrance. The opened end is sealed with nylon fabric before the immersion to prevent escape of larvae. Change adopted by Silva et al. (2011) consisted of increasing time of larvae immersion from 30 seconds to five minutes. The method described by Sindhu et al. (2012) adds the standard volume of two milliliters of test solution into syringes of 3 mL containing the larvae. Test solution is discarded by pushing the plunger up to the gauze, eliminating the need for air exhaust orifice.

Immersion Tests were carried out with *R. (B.) microplus* kept on cattle free of hemoparasites at Embrapa South Livestock. All animal procedures were approved by the Santa Catarina State University Institutional Animal Care and Use

Committee. Engorged females were stored in incubator at 27 °C and 80% relative humidity (RH) and eggs transferred to 20 mL syringes. Groups of 100 active larvae, from 10 to 15-day-old, were transferred to syringes tests 24 hours before the bioassay and syringes incubated in the dark at 27 °C and 80% RH. A system that connects the syringe test to oil-less vacuum and pressure pump was developed to transfer larvae to syringe test and to count larvae at the end of exposure time (Figure 1). Methods were compared exposing larvae to a chemical composition of cypermethrin (15%), chlorpyrifos (25%) and piperonyl butoxide (1%) (Texvet® - Agroline) prepared in nine serial dilutions (100, 25; 6.25; 1.56; 0.39; 0.19; 0.09; 0.02 and 0.006%). The highest concentration was prepared as recommended by the manufacturer (1 mL:1 Liter or 150 ppm of cypermethrin; 250 ppm of chlorpyrifos). Each treatment consisted of six replicates.

After larvae exposure to solutions, syringes were dried on paper towels, kept for one hour in fume hood and transferred to a room at 27 °C and 80% RH. Percent mortality was calculated to each concentration 24 hours later, considering as dead the larvae without motility. Results were submitted to analysis of variance followed by the comparison of means through randomized blocks. Percent mortality and median lethal concentration ( $LC_{50}$ ) of each treatment was corrected by control means groups, exposed to distilled and deionized water, according to (ABBOTT, 1925).  $LC_{50}$  was calculated in GraphPad Prism (V. 6.05, 2014) after transforming acaricide concentrations in logarithm ( $x = \log(x)$ ). Slope of the dose-response curve was defined as variable, and maximum and minimum mortality defined as hundred and zero, respectively.

**Figure 1.** Vacuum and pressure system used for transferring storage *Rhipicephalus (Boophilus) microplus* larvae to syringes test (A) and to count larvae at the end of exposure time in Larvae Immersion Test (B). The vacuum or pressure pump function was alternated to suck or expel larvae, as needed during the bioassay.

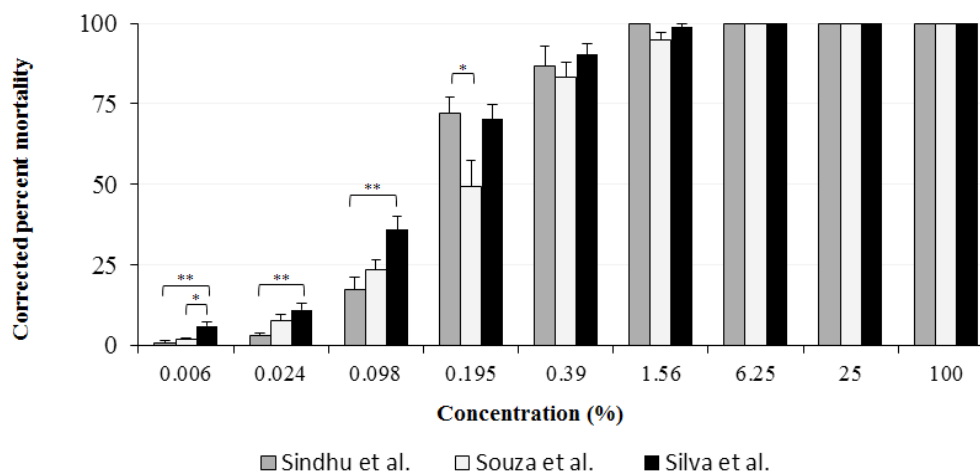


Source: (ALVES, 2014).

Larvae mortality was directly proportional to increase of acaricidal concentration for the three methods (Figure 2). Control mortality did not exceed 5% in all treatments. Mortality in distilled and deionized water was higher in Silva et al. (2011) method ( $1.48\% \pm 0.79$ , mean  $\pm$  SE), followed by Sindhu et al. (2012) ( $0.57 \pm 0.41$ ) and Souza et al. (2008) ( $0.33 \pm 0.17$ ). Mortality

of larvae exposed to chemical dilutions on Silva et al. (2011) were significantly higher than other methods at lower concentrations (Figure 2), probably in consequence of longer exposure time of larvae to solutions. Values of  $LC_{50}$  were 0.125%, 0.153% and 0.186% for Silva et al. (2011), Sindhu et al. (2012) and Souza et al. (2008) methods, respectively.

**Figure 2.** Acaricidal activity of chemical solutions containing cypermethrin and chlorpyrifos on larvae of *Rhipicephalus (Boophilus) microplus* evaluated by Larvae Immersion Techniques in syringes described by Souza et al. (2008), Silva et al. (2011) and Sindhu et al. (2012).



\* p < 0.05; \*\* p < 0.01.

Changes proposed by Silva et al. (2011), increasing larva immersion time from 30 seconds to 5 minutes, lead to lower  $LC_{50}$ . Sindhu et al. (2012) modifications make the test safer and more practical since (i) reduces syringes preparation time, (ii) reduces space required for syringe storage (the 3 mL syringe is used instead of a 5 mL), (iii) decreases the risk of environment and the handler contamination with the test solution (volume prepared is reduced and discharge process is cleaner) and (iv) saves solution (only 2 mL of solution by replication).

From the three evaluated methods, the use of 3 mL syringes without an orifice, 30 seconds immersion time and the standard of two milliliters of test solution into each syringe make larval immersion test in syringe less laborious, without losing results reliability.

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**This study was approved by the Ethics Committee on Animal Experiments (CETEA) of the University of Santa Catarina State (UDESC) under protocol number 01.59.14.**

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