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Determination of LC_{50} of permethrin acaricide in semi-engorged females of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae)

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ABSTRACT

Currently, the most efficient and widely used method of tick control still is the treatment with acaricides, especially permethrin (active ingredient of the Advantage® Max3, Bayer), a pyrethroid with neurotoxic action. Due to the wide use of this acaricide in the control of the tick *Rhipicephalus sanguineus*, this study carried out laboratorial procedures to determine the LC_{50} (lethal concentration fifty) of permethrin in semi-engorged females of *R. sanguineus*. Based on the result of 14 dilutions of permethrin in distilled water and later Probit analysis, the LC_{50} of permethrin for *R. sanguineus* was 2062 ppm (1549–2675 ppm). This work can be used as a protocol with other chemicals, to determinate the LC_{50}, basic procedure for studies of control, resistance and behavior of ticks treated with acaricides, especially the brown dog tick *R. sanguineus*. Also, the knowledge of the LC_{50} provides information on the potency of chemicals, the sensitivity of Arthropods to them and even estimates on pest control.

1. Introduction

The brown tick *Rhipicephalus sanguineus* (Latreille, 1806) is an ectoparasite of dogs of great medical and veterinary importance, which produces debilitating effects due to blood losses in affected animals and transmission of several pathogens such as *Ehrlichia canis*, *Babesia canis*, *Haemobartonella canis* and *Hapatozoon canis* (Borges et al., 2007).

Numerous studies are currently under way to find an effective control strategy that would minimize the damage caused by these parasites. A new tick control approach is an immunological one, consisting in the identification, isolation and synthesis of proteins that protect tissues and organs of the tick, mainly those of the reproductive system, with the aim of developing a vaccine (Tellam et al., 1992; Willadsen, 1997). However, nowadays the most efficient method to control tick populations is by using chemical compounds, such as permethrin acaricide, a synthetic pyrethroid that acts in the nervous system of the ticks (Mencke et al., 2003).

The toxicity of an acaricide is defined as extent or degree to which a chemical substance to kill or injure the target pest. In this way, the toxicity of a drug is determined by running laboratorial tests on animals and it is expressed as LD_{50} (lethal dose fifty) and LC_{50} (lethal concentration fifty) values and are the amount or concentration, respectively, of the pesticide's active ingredient that is required to kill 50% of the tested animals under standardized tests conditions (Garcia-Garcia et al., 2005).

Thus, due of the widespread use of permethrin (active ingredient of the Advantage® Max3, Bayer) in acaricides, this study described for the first time a detail protocol of laboratorial procedures to determinate the LC_{50} of permethrin in semi-engorged females of *R. sanguineus*, since few descriptions of adult immersion tests (AIT) are related in the literature and some of them are very old (Whitnall and Bradford, 1947; Hitchcock, 1953; Drummond et al., 1973).

2. Material and methods

2.1. Ticks

In the present study, were used throughout the experiment a total of 240 semi-engorged females (mean weigh ± SD = 27 mg ± 4.4) of *R. sanguineus* that had never been treated with acaricides. The individuals which did not present mutilations or malformations were selected under the Motic SMZ 168 TL stereoscope. The ticks were supplied by the colony maintained at the Brazilian Central of Studies on Ticks Morphology (BCSTM) at the Universidade Estadual Paulista "Júlio de Mesquita Filho", UNESP, campus of Rio Claro, SP,

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Brazil, under controlled conditions (28 ± 1 °C, 80% humidity and 12 h photoperiod) in BOD (Biological Oxygen Demand) Eletrolab EL 202 incubator and fed on New Zealand white rabbits (Approved by Comitê de Ética em Pesquisa e Mérito Científico—UNIARARAS, Protocol n° 011/2009).

The feeding laboratorial conditions of *R. sanguineus* ticks in the hosts were carried out according Bechara et al. (1995).

2.2. Chemical compound

Permethrin acaricide (3-phenoxybenzyl(1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, $C_{21}H_{20}Cl_2O_3$) (CAS n°: 52645-53-1) used in this study was purchased by Fersol Indústria e Comércio S/A (Mairinque, SP, Brazil).

2.3. Adult immersion tests (AIT)

2.3.1. Bioassay 1

To determinate the mortality interval of ticks, permethrin was diluted in distilled water in order to prepare immersion solutions and tested at the following concentrations: 384000 ppm (commercial product—not diluted), 38400, 25600, 3840, 2560, 1536, 1024, 512, 256, 51.2 and 25.6 ppm. The solutions were homogenized by shaking. The ticks of control group were exposed only to distilled water. For each concentration and control group was used 5 semi-engorged females. All treatments were tested in duplicates (10 individuals for each treatment group).

Before the tests, the females of *R. sanguineus* were washed in a sieve with tap water and were dried on soft absorbent paper. After that, the ticks were subjected to the treatments groups and immersed in Petri dishes for 5 min containing the different permethrin concentrations. The control group ticks were immersed in distilled water for the same period. Ticks were then dried in absorbent paper and placed into identified Petri dishes in a BOD incubator (28 ± 1 °C, 80% humidity and 12 h photoperiod) for 7 days. This period was suggested by Oliveira et al. (2008) in studies with *R. sanguineus* females subjected to another chemical product. The observation period was established because most often the effect of the acaricide is not immediate, because it acts slowly on the physiology of the individual analyzed.

Only ticks' females capable of locomotion were considered alive.

2.3.2. Bioassay 2

After the detection of the mortality interval, the permethrin acaricide was again diluted in distilled water and tested in another series of dilutions spaced at small intervals: 3491, 3036, 2560, 2048, 1536 and 1024 ppm. These values are inside of the mortality interval previously established in the **bioassay 1**.

The same procedures of wash in tap water and immersion of the ticks in the different concentrations of acaricide described in the **bioassay 1** were adopted, including the treatment made in the control group. Then, the ticks were maintained in a BOD incubator (28 ± 1 °C, 80% humidity and 12 h photoperiod) for 7 days. For each concentration as well as in control group were used 5 semi-engorged females of *R. sanguineus* ticks. These treatments were tested in triplicates (15 individuals for each group). In this bioassay a larger number of individuals was used so that the mortality data were subjected to Probit analysis.

2.3.3. Probit analysis

The Probit analysis was used due to need to quantify the responses obtained in bioassays using live organisms (test target), in this case females of the tick *R. sanguineus*. Ticks were exposed to a stimulus (different concentrations of permethrin), resulting in a binary response corresponding to death or survival of treated individuals. This was a specific case of a binary response bioassay with one explanatory variable: different doses of permethrin.

In this way, the results obtained in the **bioassay 2** were subjected to Probit analysis with the POLO-PC software (LeOra Software, 1987) to calculate the LC_{50}, slope and 95% confidence interval (Haddad, 1998) of permethrin in semi-engorged females of *R. sanguineus*.

3. Results

The results of the **bioassays 1** and **2** show 0% mortality and no changes in behavior of *R. sanguineus* females of the control group. However, at the concentration of 384000 ppm, mortality is observed soon after the onset of treatment of individuals. At the concentrations of 38400 and 25600 ppm, a progressive increase in mortality is observed throughout the observation period (Table 1).

When females are treated with higher permethrin concentrations (384000, 38400 and 25600 ppm) they show reactions such as excitation, repetitive movements, progressive decrease in locomotor activity, shivering and debilitation, stretching of all legs and paralysis that may or not be followed by death, besides the integument exhibit appearance dehydrated (shriveled). For the remaining concentrations tested, these effects are less intense or not observed.

At the concentrations 3840, 3491, 3036, 2560, 2048, 1536, 1024 and 512 ppm of permethrin, mortality rates decrease as concentrations decrease and 100% mortality is not observed. In addition, when an individual dies, the process is not immediate, leading to a slow death in females more sensitive to permethrin.

In the group of females treated with permethrin at the concentrations of 256, 51.2 and 25.6 ppm 0% mortality and any external morphological alterations (dehydrated integument) or behavioral changes were observed.

Thus, the results obtained in the **bioassay 1** show that the mortality interval of *R. sanguineus* females is between 512 and 3840 ppm. Based on these results, in **bioassay 2**, the percentage of dead individuals after exposure to concentrations of permethrin was quantified (Table 2). These data were then subjected to Probit analysis, a mathematical model that determined that the LC_{50} of permethrin for semi-engorged females of the *R. sanguineus* is 2062 (1549–2675 ppm) (Table 3).

4. Discussion

Permethrin is a topical acaricide absorbed by the tick's integument, which produces a series of uncoordinated nerve impulses as a result of changes in the permeability of membranes to sodium—repetitive effect. Consequently, sensory organs and nerve terminations are especially sensitive, triggering a state of excitation followed by paralysis and death of parasites (Mencke et al., 2003).

Thus, due of the widespread use of permethrin in acaricides, this study described for the first time a detail protocol of laboratorial procedures to determinate the LC_{50} of permethrin in semi-engorged females of *Rhipicephalus sanguineus*.

Descriptions of larval and adult immersion tests (LIT and AIT, respectively) in acaricides are limited and some of them are very old (Whitnall and Bradford, 1947; Hitchcock, 1953; Shaw, 1966; Drummond et al., 1973). In this way, modifications in these previous tests also have been made to optimize the technique (Sabatini et al., 2001; White et al., 2004; Klafke et al., 2006; Jonsson et al., 2007). There is, however, no standard protocol for AIT by Food and Agriculture Organization (FAO).

In the present study, AIT in permethrin was 5 min, due rapid topical activity of this chemical (White et al., 2004). However, the time of immersion is a critical point in AIT. Drummond et al.