

# DETERMINATION OF THE MINIMUM IMMERSION TIME OF *BOOPHILUS MICROPLUS* (CANESTRINI, 1887) ENGORGED FEMALES FOR *IN VITRO* RESISTANCE TESTS WITH AMITRAZ AT 50% EFFECTIVE CONCENTRATION (EC<sub>50</sub>)

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**SUMMARY:** Immersion tests were conducted with engorged females of the cattle tick *Boophilus microplus* in order to determine the minimal immersion time for use in immersion tests with the acaricide Amitraz at the effective concentration 50% (EC<sub>50</sub>). The tick strain used - the Mozo strain - can be assumed as a reference strain due its susceptibility to the acaricides currently used. Groups with 10 engorged females each were immersed into different concentrations of pure compound diluted in acetone (40%), for 1, 5, 10 or 25 minutes. After incubation and hatching, hatching percentage was assessed and percentages of product efficacy calculated. The minimal immersion time determined was 5 minutes, as stated by the analysis of confidence limits (95%). This EC<sub>50</sub> value could be used as reference for the assessment of resistance index, once it had been stated in a sensible strain.

**KEY WORDS:** Resistance tests, *Boophilus microplus*, amitraz.

## INTRODUCTION

Tick resistance has regularly followed the use of acaricides for the control of *Boophilus microplus* (MENDES *et alii*, 2000; BEUGNET & CHARDONNET, 1995; OLIVEIRA *et alii*, 1986 and NOLAN, 1989). Many ways to handle this problem were developed, and among them the *in vitro* diagnosis and screening tests appear to be the most indicated (DRUMMOND *et alii*, 1973). Such methodologies allow us to identify the resistance status in the field, which is a very desirable trait, since that once susceptibility is lost it seldom can be restored. Early diagnosis is therefore needed if management of resistance is to be intended. One of the most widely used technique is the immersion of engorged females in the suspected acaricide, with immersion times chosen in an arbitrary way. Based on previous data obtained in tests with other acaricides (MENDES *et alii*, 2000), we conducted the present work to determine the minimal immersion time of engorged females for the 50% effective concentration (EC<sub>50</sub>) with the acaricide amitraz, using different immersion times (1, 5, 10 and 25 minutes).

## MATERIALS AND METHODS

### Tick Strain

The strain used in this test, the Mozo strain, has been kept in laboratory at the Department of Parasitology - ICB/USP since 1994. Due to its susceptibility to the acaricides currently used, it can be assumed as a reference strain (MENDES *et alii*, 2000). The engorged females used in this test were allowed to grow in donor calves and collected after natural fall. They were washed, dried, and then sorted by color and weight. Females showing any damage were discarded.

### Immersion tests

Replicates were made with groups composed by 10 engorged females each. Three groups were allocated to each treatment. They were immersed into different concentrations of pure compound diluted in acetone (40%), for 1, 5, 10 or 25 minutes. The concentrations used are presented in Table 1. Controls were dipped in acetone only. After immersion, the females were allowed to dry and placed in Petri dishes (one for each group)

Table 1 - Distribution of experimental groups used in the determination of the Amitraz  $EC_{50}$ . All solutions diluted in acetone (40%).

Experimental group	Concentration of Amitraz	Number of replicates	Number of engorged females/replicate
A	50	3	10
B	25	3	10
C	10	3	10
D	2	3	10
E	1	3	10
F	0.8	3	10
control	0	3	10

and kept on incubator (B.O.D.) at 27°C and 85% relative humidity to allow oviposition, after what the eggs were weighted, placed in test tubes and returned to the incubator until hatching. The percentage of hatching was recorded. The results were assessed by measuring the effect over the tick reproduction capability, and shown as percentage of product efficacy. Briefly, the inhibition of egg-laying was calculated based upon the egg weight data, using the following formula (DRUMMOND *et alii*, 1973):

$$\% \text{ Inhibition} = \frac{\text{Weight of Eggs of the Treated Group} \times \text{Weight of Females of the Control Group}}{\text{Weight of Females of the Treated Group} \times \text{Weight of Eggs of the Control group}}$$

The Reproductive Efficiency (RE) and the Product Efficacy (PE) were assessed in agreement with DRUMMOND *et alii* (1973). The PE data was then transformed by means of probit analysis.

## RESULTS

The values found for the  $EC_{50}$  were: 1,12 ppm for 1 minute, 0,907 ppm for 5 minutes, 0,86 ppm for 10 minutes and 0,905 ppm for 25 minutes (Table 2). The probit transformed data for the different times are represented in the figures 2 to 5. The minimal immersion time determined was 5 minutes, as stated by the analysis of confidence limits (95%) obtained through the probit method presented in the Figure 1.

## DISCUSSION

Based on these results, it is suggested that 5 minutes should be adopted as the standard minimal immersion time for tests with engorged females when Amitraz is used. This conclusion is based upon the observation of the data presented in the Figure 1, where it can be verified that the value of the confidence limit for the immersion time of 5 minutes comprehends all the range of possible values for the  $EC_{50}$  of both 10 and 25 minutes. In fact there is none significant variation between the  $EC_{50}$  found values for these 3 times. So, one can choose 5 minutes as the standard minimal immersion times for practical reasons. This

Table 2 -  $EC_{50}$  values for the immersion times of 1, 5, 10 and 25 minutes, determined by probit analysis.

Immersion time	$EC_{50}$ (ppm)	Confidence limits	
		Max.	Min.
1 min.	1.1234	1.4931	0.8356
5 min.	0.9077	1.2656	0.6117
10 min.	0.8670	1.1226	0.6632
25 min.	0.9050	1.2457	0.6479

$EC_{50}$  value could be used as reference for the assessment of resistance index, once it had been stated in a sensible strain.

It is also worth-while to mention the adequacy of the probit method, especially the  $EC_{50}$  95% confidence limits, for the analysis of effective concentration data. It presents results in a easy-to-see way, as can be confirmed in Figure 1.

The results found here are in agreement with the data of MENDES *et alii*, 2000, who worked with pyrethroid acaricides, and also found immersion times of 5 and 10 minutes. For some acaricides (Deltamethrin and Cifluthrin) the  $EC_{50}$  95% confidence limits profile resembled the profile found here for Amitraz, *i.e.* the times of 5, 10 and 25 minutes presented closer values, and the 5 minutes time was chosen for time-saving reasons.

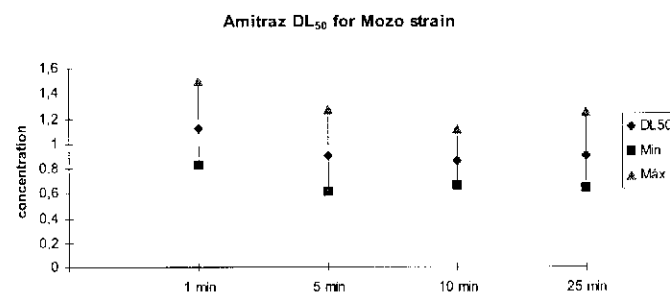


Figure 1 - Confidence limits of  $EC_{50}$  (95%) for the acaricide Amitraz with Mozo strain. Data obtained by Probit analysis.

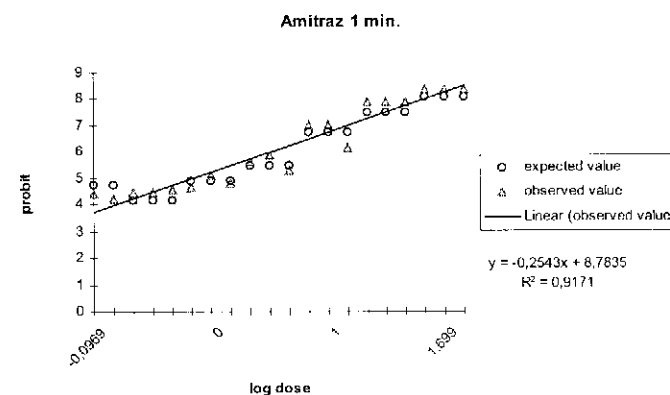


Figure 2 - Efficacy of the acaricide Amitraz for Mozo strain for the 1 minute immersion time. Product efficacy data transformed by Probit analysis.

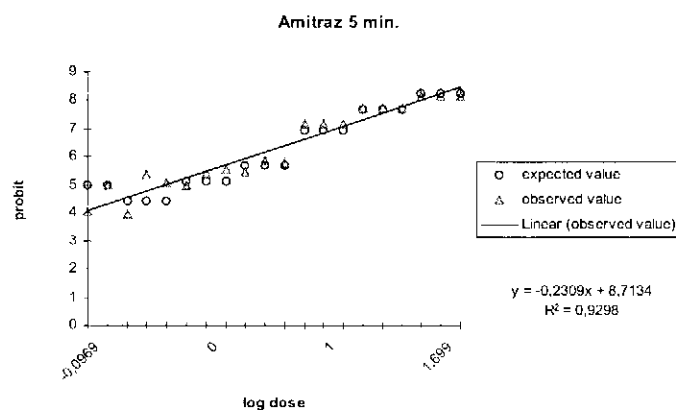


Figure 3 - Efficacy of the acaricide Amitraz for Mozo strain for the 5 minutes immersion time. Product efficacy data transformed by Probit analysis.

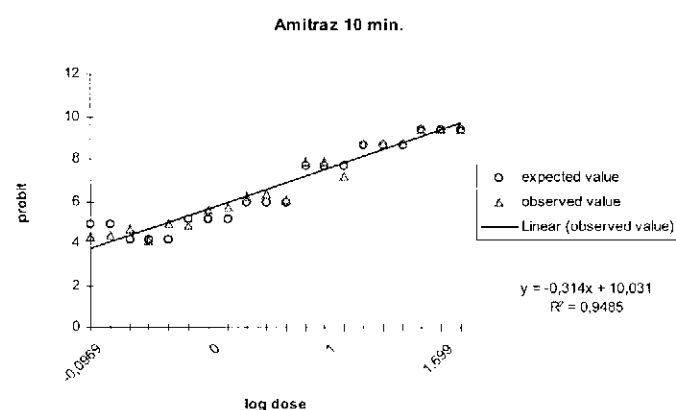


Figure 4 - Efficacy of the acaricide Amitraz for Mozo strain for the 10 minutes immersion time. Product efficacy data transformed by Probit analysis.

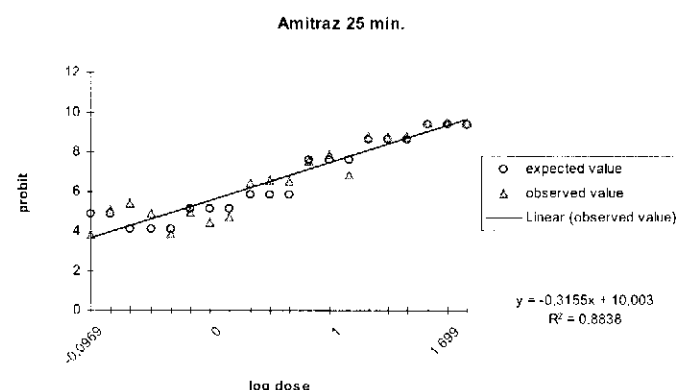


Figure 5 - Efficacy of the acaricide Amitraz for Mozo strain for the 25 minutes immersion time. Product efficacy data transformed by Probit analysis.

## SUMÁRIO

Testes de imersão foram realizados com teleóginas de *Boophilus microplus* a fim de determinar o tempo mínimo de imersão a ser usado em testes com o acaricida Amitraz, na concentração eficaz a 50% (CE<sub>50</sub>). A cepa de carrapato utilizada - a cepa Mozo - pode ser considerada como cepa padrão devido à sua susceptibilidade aos acaricidas em uso. Grupo com 10 teleóginas cada foram imersos em diferentes concentrações de produto puro diluído em acetona (40%), por 1, 5, 10 ou 25 minutos. Após a incubação e eclosão, a percentagem de eclosão foi medida e o percentual de eficácia do produto calculado. O tempo mínimo de imersão determinado foi o de 5 minutos, estabelecido pela análise dos limites de confiança a 95%. O valor da CE<sub>50</sub> obtido poderá ser usado como valor de referência para o cálculo do fator de resistência, uma vez que foi estabelecido em uma cepa suscetível.

**PALAVRAS-CHAVE:** Testes de resistência, *Boophilus microplus*, amitraz.

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