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In Vitro and *in Vivo* Efficacy of an Experimental Compound against *Rhipicephalus* (*Boophilus*) *microplus* Ticks

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ABSTRACT

The aim of the present study was to evaluate the ixodicide efficacy of the experimental compound 712-BF-016 against *Rhipicephalus* (*Boophilus*) *microplus* ticks *in vitro* and in cattle. The *in vitro* efficacy was initially tested against R. *Boophilus microplus* larvae using the Larval Packet Test (LPT). In a 2nd study the ixodicide efficacy was tested against adult ticks using the Adult Inmersion Test (AIT). Finally, a field test with the compound was carried out using 24 steers experimentally infested with *R.* (*Boophilus*) *microplus* ticks which were divided into 4 groups of 6 animals each for treatment. Groups 1 and 2 received the experimental compound at concentrations of 16% and 20%, respectively, which were applied as an aspersion in a total volume of 4 liters/animal. Group 3 was equally treated but with a commercial ixodicide containing cipermethrin at a 16% concentration. Group 4 served as untreated control. The efficacy was measured on days 1, 2, 3 after treatment as the percentage of ticks present from the treated groups, relative to the ticks present in the untreated control. The results indicated a percentage mortality of 93.21% for LPT and 98.02% for AIT. The efficacy produced in cattle was 61.78%, 76.43% and 85.34% for groups 1, 2 y 3, respectively. It is concluded that there was no concordance between the results obtained *in vitro* with those found in cattle. Possibly the excipient used for the formulation of the experimental compound was not suitable and had some influence on the results.

Keywords: *Rhipicephalus Boophilus microplus*; Ixodicide; LPT; AIT; Cattle

1. Introduction

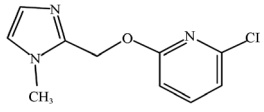
The cattle tick *Riphicephalus* (*Boophilus*) *microplus* is one of the most important ectoparasites because it causes economical losses of several million dollars to the world economy [1].

In Mexico, among the 77 especies of ticks identified, *R.* (*Boophilus*) *microplus* and *Amblyoma cajennense* are the most oustanding for cattle owners [2]. Ref. [3] mentioned that *R. Boophilus microplus*, present in 53% of the national territory, is distributed mainly in low tropical areas.

The control of this tick in Mexico is based on the use of ixodicides [4,5]; however, nowadays a considerable number of them produce resistance due to excesive and inadequate use.

Although several tests to determine the suceptibility to a particular acaricide are available, the Adult Inmersion Test (AIT) [6] is the test of worldwide reference which is approved by FAO for the determination of resistance [7]. Another test frequently employed is the Larval Paquet Test (LPT) [8], which determines the percentage of mortality in larvae treated with therapheutic concentrations of an acaricide.

Very recently our group from the Chemistry School of the National Autonomous University of Mexico (UNAM), has sinthesized a compound coded 712-BF-016 which was selected among 85 other molecules having the following characteristics: White powder, Molecular weight 223.66, melting point 48˚C - 50˚C, soluble in EtOH, DMSO, C_6H_6, AcOEt, Acetone, Trichloroethane and slightly soluble in water. Its molecular formula is: $C_{10}H_{10}CIN_{30}$.



Chemical structure.

The present study was aimed at evaluating the ixodicide efficacy of compound 712-BF-016 against *Rhipicephalus* (*Boophilus*) *microplus* ticks *in vitro* and in cattle.

2. Material and Methods

2.1. Location of the Study

All *in vitro* tests were carried out at the Parasitology Department of the Veterinary School and the field study in cattle was undertaken at the "Santa Cruz" ranch located at Ayotoxco, Puebla (southeastern part of Mexico).

2.1.1. First Experiment (Larval Packet Test (LPT)) [8]

Five discriminant doses of the experimental compound (1%, 0.50%, 0.25%, 0.13%, 0.06%) were prepared for the treatment groups and an untreated control group (using 30 ml of vegetal oil and 60 ml of trichloroetilene) was included. For the larval packets Whatman No. 1 filters (7.5 × 8.5 cm) were used, the name of the compound being identified for each dilution. Then papers were impregnated with the compound dilutions and each dilution was carried out three times.

The packets were bent with the impregnated face on the inside site. Finally the free ends were sealed with a paper pressing holder.

With the aid of a small paintbrush taking approximately 100 fourteen-day old larvae were placed inside the packet. All packets were incubated at 28˚C ± 2˚C for 24 hours with an 80% - 90% relative humidity. With the aid of a table counter dead and live ticks from each packet were counted to estimate the response percentages of mortality for each dilution.

Parameters evaluated. To calculate the Mortality Index due to the effect of the experimental ixodicide and the larval survival, the following formula was applied: [4].

$$\%\ \text{larval mortality} = \left(\text{Dead larvae}/\text{Total number of larvae}\right)\times 100$$

To calculate the average mortality, the following formula was employed:

$$\text{Mean percentage of mortality} = \frac{\left(\text{mortality1}+\text{mortality2}\right)}{2}$$

2.1.2. Second Experiment Adult Inmersion Test (AIT) [6]

Five discriminant doses were prepared with the experimental compound (1%, 0.50%, 0.25%, 0.13% y 0.06%) leaving one untreated control group.

The doses were then placed in individual beakers, and 10 adult ticks were immersed in each of the beakers for 30 minutes. Five repeats were carried out for each dose and the control group was only immersed in distilled watter.

After the ticks were sieved and dried with paper towels , they were then stuck on the dorsal face with scotch tape after being placed in a Petri dish. The ticks were then incubated at 28˚C ± 2˚C and 80% - 90% relative humidity for seven days. After incubation, the number of ticks which ovoposited per group were counted. In order to obtain the complete oviposition the ticks were then incubated for another 7 days and the percentage of mortality and the mean weight of ovigerous-mass were obtained.

Efficacy measurement. The efficacy was calculated by means of the formula (see the bottom of the page) [10].

2.1.3. Field Test in Cattle

Twenty-four crossbred steers with an average weight of 200 kg were used. Each animal was infested with 40,000 susceptible larvae of *R*. (*Boophilus*) *microplus* obtained from 2 young cattle previously infested with ticks. Twenty-one days after the infestation, the engorged ticks present were counted, the animals having been divided into 4 groups (G) of 6 animals each for treatment.

- G1 received a single aspersion with the experimental compound formulated at 16%. It was applied with the aid of an aspersion bomb in a total volume of 4 liters/animal.
- G2 was treated in a manner similar to that of G1 but the concentration of the experimental compound was 20%.
- G3 treated with a commercial ixodicide (Tlalaxin-Shark®) containing Cipermethrin at 16%, was used as a drug of reference.
- G4 was an untreated control.

Percentage of ticks that oviposited [11].

$$\%\ \text{of ovipositing ticks} = \frac{\text{Number of ovipositing ticks}\times 100}{\text{Total number of female ticks}}$$

The oviposition index was calculated by the following formula: [11].

$$\text{Oviposition Indes} = \frac{\text{Weight of eggs}\left(\text{g}\right)\left(20,000\right)}{\text{Weight of female ticks}\left(g\right)}$$

where: 20,000 = Number of larvae present in 1 gram of eggs.

$$\text{Efficacy} = \frac{\text{Mean number of control ticks} - \text{Mean number of treated ticks}\times 100}{\text{Mean number of control ticks}}$$

C.F. of % of hatching = Centesimal fraction of the % of hatching.

The percentage of hatching inhibition was calculated according to [11].

$$\% 10 = \frac{(OP/T - OP/t)}{OP/T}(100)$$

where:

OP/T = Estimated reproduction of the control group.

OP/t = Estimated reproduction of the experimental group.

The percentage of hatching was established as follows: [10].

$$\% \text{ Hatching} = C/E + C \times 100$$

where: C = eggshells, E = eggs.

Finally the efficacy was calculated as above mentioned with the *in vitro* tests.

3. Results and Discussion

In vitro percentage of larval mortality (LPT).

Table 1 shows that the more incremented were the concentrations with the experimental compound higher percentage of mortality was obtained. Here concentrations at 1% produced a 91.1% of mortality while the average mortality percentage was 91.6.

However, when the other tested concentrations were analyzed, it was observed that the efficacy decreased gradually and the percentages of efficacy came down to considerably low levels.

The statistical analysis indicated important differences among treatments, the pattern being repeated in the case of average mortality. There are significant differences between treatments including the control group.

Table 2 shows that as the experimental compound was tested against adult ticks (AIT), the exerted efficacy measured as a percentage of mortality was high (97.65%) at a concentration of 1%, the efficacy gradually decreasing with the subsequent concentrations. Here it is important to note that in most of the decreased concentrations, the efficacy remained constant with values close to the 1% concentration, (95.54%, 91.98%, 85.94% and 77.51%), thus showing a better constant effect than that obtained with the larval packet test (LPT).

Statistical differences in efficacy were detected at all concentrations, except at 1 and 0.5%.

With regard to the mean weight of eggs from adult treated ticks, no differences were determined at 1 and. 50% concentrations but the rest showed to be statistically different. (**Table 2**).

Results obtained in the field test with cattle, where the exerted efficacy was of 61.78% for the experimental compound formulated at a 16% concentration and 76.4% for the formulation prepared at a 20% concentration, demonstrated that the commercial compound containing a 16% cipermethrin showed an 85.3% efficacy (**Table 3**).

The statistical analysis showed that the mean number of ticks before and after the treatment indicated significant differences among the given concentrating the most being efficient cipermethrin at 16%. (**Table 3**).

Table 1. *In vitro* Percentage of larval mortality of *Riphicephalus Boophilus microplus* with the experimental compound 712-BF-016 by mean of the Larval Packet Test.

Concentration	Alive	Dead	Total	% Mortality	Average % Mortality
Untreated Control 1	100	0	100	0	0
Untreated Control 2	100	0	100	0	0
A1 (1%)	10.4	136.6	147	93.1[e]	91.63[e]
B1 (0.5%)	22.4	123	145.4	84.5[d]	80.29[d]
C1 (0.25%)	50.2	90.8	141	63.9[c]	58.5[c]
D1 (0.125%)	72	65.8	137.8	45.1[b]	42[b]
E1 (0.0625%)	93.8	36.4	130.2	24.1[a]	26.5[a]

[a,b,c,d,e]Different superscripts indicate statistical difference ($P < 0.05$).

Table 2. *In vitro* Percentage Mortality of adult *Riphicephalus* (*Boophilus*) *microplus* with the experimental compound 712-BF-016.

Concentrations	1%	0.50%	0.25%	0.13%	0.16%
Percentage of efficacy	97.65[a]	95.54[a]	91.98[b]	85.94[c]	77.51[d]
Average weight of eggs from adult treated ticks with the experimental compound 712-BF-016					
	0	0.01	0.19	0.32	0.37
Control (water)	0.00887[a]	0.0136[b]	0.00732[c]	0.00972[d]	0.00754[c]

[a,b,c,d]Different superscripts indicate statistical difference ($P < 0.05$).

Abbott (1925)

Table 4 shows that even when the 20% formulation gave on apparently better performance, it was observed that when the data of the Oviposition Index (0.28) and the Percentage of the Inhibition of the Oviposition (50.1) were related, the results indicated that the formulation of the experimental compound applied at 16% was better since this group had a better effect on mortality.

When the oviposition index and the percentage of inhibition of oviposition were compared with the concentrations of 16% of compound 712-BF-016 and 16% cipermenthrin no important differences were noted. However, the percentage of inhibition showed statistical differences when the three treatment-concentrations were analyzed (**Table 4**).

With regard to the percentage of hatching from tick-eggs collected from the steers after the treatment, the information revealed that the lower percentage of egg hatching per group was obtained from the 16% experimental formulation (50.57%) and the highest percentage of tick egg hatching was with that of 16% cipermethrin (74.89%). This demonstrates that under these circumstances cipermethrin did not have a high effect on the hatching of the treated tick-eggs.

Looking at the percentage of hatching, significant differences were observed in all evaluated groups (**Table 5**).

It can be said generally that the efficacy exerted by the experimental compound under *in vitro* conditions proved to be satisfactory, particularly against adult ticks. However, the efficacy exerted by the experimental compound under field conditions in cattle showed neither a similar correlation or a similarity with the *in vitro* results. Possibly the excipient used to formulate the experimental compound was not suitable and therefore the efficacy obtained was lower.

Further studies should be conducted using different excipients aimed at determining whether the ixodicide efficacy of the experimental compound can increase or not.

Table 3. Percentage efficacy of compound 712-BF-016 and Cipermethrin against *Riphicephalus* (*Boophilus*) *microplus* ticks in artificially infested cattle.

Groups	Drug concentration	Mean number of ticks before treatment	Mean number of ticks after treatment	Efficacy %
1	712 FB 016 at 16%	81.83[a]	32.16[a]	61.78[a]
2	712-BF-016 at 20%	33.33[b]	19.83[b]	76.43[b]
3	cipermethrin 16%	35.66[b]	12.33[c]	85.34[c]
4	untreated control	84.16[c]	73.16[d]	-

[a,b,c,d]Different superscripts indicate statistical difference ($P < 0.05$).

Table 4. Oviposition Index and Percentage inhibition of the oviposition of collected ticks from cattle treated with compound 712-BF-016 or with Cipermehtrin.

Groups	Drug concentration	% of oviposited ticks/group	Oviposition Index	% inhibition of the ovoposition
1	712-BF-016 at 16%	12.19	0.28[a]	50.1[a]
2	712-BF-016 at 20%	10.97	0.34[b]	39.55[b]
3	Cipermethrin 16%	9.18	0.24[a]	57.58[c]
4	Untreated control	11.57	0.57[d]	-

[a,b,c,d]Different superscripts indicate statistical difference ($P < 0.05$).

Table 5. Hatching percentage of eggs from *Riphicephalus* (*Boophilus*) *microplus* ticks collected from cattle treated with compounds 712-BF-016 or cipermethrin.

Groups	Drug concentration	Mean number of eggs	Mean number of eggshell	Hatching percentage
1	712-BF-016 at 16%	66.73	55.53	50.57[a]
2	712-BF-016 at 20%	35.37	31.97	55.99[b]
3	Cipermethrin at 16%	15.57	26.63	74.89[c]
4	Untreated control	11.77	70.89	89.14[d]

[a,b,c,d]Different superscripts indicate statistical difference ($P < 0.05$).

4. Conclusion

The experimental compound 712-BF-016 evaluated against *Riphicephalus* (*Boophilus*) *microplus* ticks showed high efficacy against larvae and adult ticks *in vitro*. However, this exerted efficacy was considerably low when a field test in cattle was performed.

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