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# Acaricidal effects of fenvalerate and cypermethrin against *Rhipicephalus (Boophilus) annulatus*

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**Abstract.** The acaricidal effects of two most commonly used acaricides *viz.*, fenvalerate and cypermethrin against *Rhipicephalus (Boophilus) annulatus* were studied using Adult Immersion Test (AIT). The $LC_{50}$ values observed for fenvalerate and cypermethrin were 1570 ppm and 184 ppm respectively. The death of ticks was not an immediate process. Fenvalerate caused death only after 7 days while cypermethrin after 5 days of treatment. The eggs laid by treated ticks did not hatch at all concentrations tested.

## INTRODUCTION

Diseases and feed scarcity are the major threats encountered by Indian cattle population (Birthal & Jha, 2005). The greater part of national economic loss due to livestock diseases is contributed by parasitic diseases, in which ticks and tick borne diseases (TTBDs) have a major share. The direct and indirect effects caused by ticks, especially the one-host ticks, are most significant, as there are increasing reports stating that one-host ticks (Khan, 1990, 1994; Sangwan *et al.*, 2000) replace multi-host ticks in the country. Even though *Rhipicephalus (Boophilus) microplus* is the major one-host tick affecting cattle in the country, *Rhipicephalus (Boophilus) annulatus* is the commonest species in South India (Jagannath *et al.*, 1979; Koshy *et al.*, 1982; Rajamohanan, 1982).

At present, control of tick and tick borne diseases (TTBDs) is mainly achieved by the wide spread use of chemical acaricides like organophosphates, carbamates, pyrethroids, BHC-cyclodines, amidines, macrocyclic lactones and benzoyl phenyl ureas (Ghosh *et al.*, 2007). Synthetic pyrethroids which are biodegradable, sufficiently stable and producing potent knockdown effect against insects include different generations of compounds *viz*, first (allethrin), second (tetramethrin, resmethrin, bio-resmethrin, bioallethrin, phenothrin), third (fenvalerate, permethrin) and fourth (cypermethrin, deltamethrin, flucythrinate, fluvalinate) (Bowman, 1999).

The widespread and indiscriminate use of chemical acaricides led to the development of drug resistance worldwide and has already been reported against almost all commercially available acaricides (Castro-Janer *et al.*, 2010). As per the report published by FAO (2004), the tick population in India has developed resistance against all the available acaricides. Recently, reports on resistance of *R. (B.) microplus* against diazinon (Kumar *et al.*, 2011) and synthetic pyrethroids (Vatsya & Yadav, 2011; Sharma *et al.*, 2012) revealed the importance of the problem in Northern India. However, no documented reports are available to understand the status of acaricidal resistance in ticks of domestic cattle of south Indian states.

Resistance to a chemical acaricide is assessed based on the resistance factor which is the quotient between $LC_{50}$ of field ticks and $LC_{50}$ of susceptible ticks (Castro-Janer *et al.*, 2009). Adult immersion test is considered as one of the suitable tests for detection of potency of drugs and resistance against chemical acaricides in ticks. In the present study, the acaricidal effects of the widely used synthetic pyrethroid compounds like fenvalerate and cypermethrin against *R. (B.) annulatus* are evaluated based on adult immersion test (AIT) in order to determine the $LC_{50}$ values of these chemicals.

## MATERIALS AND METHODS

### Ticks

Fully engorged adult *Rhipicephalus* (*Boophilus*) *annulatus* were collected from infested cows of a single household of "Mamalakunnu" near Meenangady, Wayanad, Kerala. These animals were not treated previously with a formulation containing fenvalerate and cypermethrin for the last one year and hence considered as 'susceptible'. The ticks were collected, washed with water, dried using absorbent paper and used for the experiment.

### Chemical compound

Pure compounds of fenvalerate and cypermethrin (purity of 99%) were obtained from AccuStandard, New Haven, CT, USA. Fenvalerate (20 mg) and cypermethrin (4 mg) were dissolved in separate beakers containing 10 mL methanol to make a 2000 and 400 ppm solutions respectively. All working dilutions were made in methanol.

### Adult immersion test

Adult immersion test (Drummond *et al.*, 1973) was used in the study for assessing three parameters *viz.*, adult tick mortality, egg weight and hatching percentage. Four replicates, each with six ticks, were used for each concentration of a single compound. Group of six ticks selected randomly based on size were weighed before the experiment and were immersed for 2 minutes in the respective dilution (10 mL) in a 50 mL beaker. Methanol was used as control. Ticks were recovered from the solution, dried using absorbent paper towel and placed in a separate plastic specimen tube (25 mm x 50 mm). These tubes were incubated in a biochemical oxygen demand (BOD) incubator at 28±1°C and 85±5 per cent relative humidity and observed for mortality. The acaricide treated ticks were observed for three parameters viz., adult tick mortality, inhibition of fecundity/egg laying (IF/IE) and hatching percentage.

Per cent of adult tick mortality within 15 days post-treatment was assessed. The weight of the egg mass laid by these ticks was recorded. Egg mass observed under the same incubation conditions in a BOD incubator for the next 30 days for visual estimation of larval hatching rate. Ticks treated with different concentrations of the two compounds were compared with the control ticks.

The index of egg laying / fecundity (IE/IF) and per cent inhibition of fecundity (%IF) were calculated as follows (Food and Agricultural Organization, 2004):

IE/IF=weight of eggs laid (mg) /weight of females (mg)

%IF = IE (control group) – IE (treated group) × 100/IE (control group)

### Statistical analysis

All data were expressed as mean ± SEM. Groups were compared using one-way analysis of variance for repeated measurements using SPSS software (IBM, USA). For *post hoc* analysis, Fishers' least squares difference and Duncan's tests were used. A value of $p < 0.05$ was considered as statistically significant.

### Probit analysis

Dose–response data were analyzed by probit method (Finney, 1952). The 50 per cent lethal concentrations ($LC_{50}$) of fenvalerate and cypermethrin against *R.* (*B*) *annulatus* were determined by applying regression equation analysis to the probit-transformed data of mortality.

## RESULTS

The acaricidal effects of fenvalerate and cypermethrin against *R.* (*B.*) *annulatus* were assessed by measuring the per cent of adult mortality, inhibition of fecundity and hatching rate. The results of the adult immersion test against *R.* (*B.*) *annulatus* are shown in Table 1 and 2.

The regression analysis and slope values of these compounds against *R.* (*B.*) *annulatus* based on probit analysis are depicted in Table 3. The $LC_{50}$ values calculated for fenvalerate and cypermethrin were 1570 ppm and 184 ppm respectively. As 100 per cent mortality could not be achieved at the highest concentration tested in the present study, the $LC_{99}$ values of these compounds could not be generated statistically. The dose-response graph based on probit analysis for cypermethrin and fenvalerate against *Rhipicephalus (Boophilus) annulatus* are represented in Figures 1 and 2.

No mortality was observed in the control group. All compounds elicited a concentration-dependent increase in adult mortality, even though 100% mortality could not be achieved in the highest concentration tested. The pattern of mortality observed

Table 1. Effects of different dilutions of fenvalerate against *R. (B.) annulatus*

| Sl. No | Acaricide | Mean ticks weight per replicate ± SEM (g) | Mean % adult mortality within 15 days ± SEM | Mean eggs mass per replicate ± SEM (g) | Index of fecundity ± SEM | Percentage Inhibition of Fecundity (%) | Hatching % (Visual) |
|---|---|---|---|---|---|---|---|
| 1. | Methanol (control) | $0.9877 \pm 0.010^{b}$ | $0 \pm 0^{a}$ | $0.5098 \pm 0.010^{c}$ | $0.5199 \pm 0.006^{b}$ | 0 | 100 |
| 2. | 1000 ppm | $1.1439 \pm 0.068^{c}$ | $33.33 \pm 6.80^{b}$ | $0.0726 \pm 0.032^{b}$ | $0.0592 \pm 0.024^{b}$ | 88.52 | 0 |
| 3. | 1250 ppm | $0.8486 \pm 0.027^{a}$ | $33.33 \pm 6.80^{b}$ | $0.0448 \pm 0.016^{ab}$ | $0.0516 \pm 0.018^{b}$ | 89.98 | 0 |
| 4. | 1500 ppm | $0.8888 \pm 0.023^{ab}$ | $49.99 \pm 6.80^{bc}$ | $0.007 \pm 0.007^{a}$ | $0.0074 \pm 0.007^{b}$ | 98.55 | 0 |
| 5 | 1750 ppm | $0.8267 \pm 0.052^{a}$ | $54.16 \pm 7.97^{bc}$ | $0 \pm 0^{a}$ | $0 \pm 0^{a}$ | 100 | 0 |
| 6 | 2000 ppm | $0.8325 \pm 0.011^{a}$ | $62.49 \pm 7.97^{c}$ | $0 \pm 0^{a}$ | $0 \pm 0^{a}$ | 100 | 0 |

n = 4, Values are Mean ± SEM, means bearing different superscripts a, b or c ($P<0.05$), indicate significant difference when compared with the control and recommended concentration of fenvalerate.

Table 2. Effects of different dilutions of cypermethrin against *R. (B.) annulatus*

| Sl. No | Acaricide | Mean ticks weight per replicate ± SEM (g) | Mean % adult mortality within 15 days ± SEM | Mean eggs mass per replicate ± SEM (g) | Index of fecundity ± SEM | Percentage Inhibition of Fecundity (%) | Hatching % (Visual) |
|---|---|---|---|---|---|---|---|
| 1. | Methanol (control) | $0.9877 \pm 0.010^{d}$ | $0 \pm 0^{a}$ | $0.5098 \pm 0.010^{d}$ | $0.5159 \pm 0.006^{e}$ | 0 | 100 |
| 2. | 100 ppm | $0.8062 \pm 0.062^{ab}$ | $24.99 \pm 10.75^{ab}$ | $0.1878 \pm 0.019^{c}$ | $0.2337 \pm 0.018^{d}$ | 54.7 | 0 |
| 3. | 150 ppm | $0.8015 \pm 0.0003^{ab}$ | $45.83 \pm 4.16^{bc}$ | $0.0628 \pm 0.023^{b}$ | $0.0784 \pm 0.029^{c}$ | 84.8 | 0 |
| 4. | 200 ppm | $0.7316 \pm 0.041^{a}$ | $45.83 \pm 14.23^{bc}$ | $0.0490 \pm 0.009^{b}$ | $0.0671 \pm 0.012^{bc}$ | 86.99 | 0 |
| 5 | 250 ppm | $0.8272 \pm 0.030^{abc}$ | $62.49 \pm 7.97^{cd}$ | $0.0508 \pm 0.025^{b}$ | $0.0591 \pm 0.029^{bc}$ | 88.54 | 0 |
| 6 | 300 ppm | $0.9149 \pm 0.026^{cd}$ | $74.99 \pm 8.33^{d}$ | $0.0164 \pm 0.010^{ab}$ | $0.0180 \pm 0.011^{ab}$ | 96.51 | 0 |
| 7 | 350 ppm | $0.8925 \pm 0.030^{bcd}$ | $74.99 \pm 10.75^{d}$ | $0 \pm 0^{a}$ | $0 \pm 0^{a}$ | 100 | 0 |
| 8 | 400 ppm | $0.9196 \pm 0.030^{cd}$ | $83.33 \pm 6.80^{d}$ | $0 \pm 0^{a}$ | $0 \pm 0^{a}$ | 100 | 0 |

n = 4, Values are Mean ± SEM, means bearing different superscripts a, b or c ($P<0.05$), indicate significant difference when compared with the control and recommended concentration of cypermethrin.

Table 3. Dose-response data of fenvalerate and cypermethrin against *R. (B.) annulatus* at 95% confidence limit

| Compound | Variables | Slope ± SE | $R^2$ | P value | $LC_{50}$ |
|---|---|---|---|---|---|
| Fenvalerate | Mortaliy | 2.675 ± 0.4909 | 0.9082 | 0.0121 | 1570 ppm |
| | Egg mass | -2.614 ± 0.5411 | 0.8537 | 0.0085 | |
| | IF | -2.632 ± 0.5680 | 0.8429 | 0.0098 | |
| | % IF | 510.0 ± 110.1 | 0.8430 | 0.0098 | |
| Cypermethrin | Mortaliy | 2.488 ± 0.2307 | 0.9588 | 0.0001 | 184 ppm |
| | Egg mass | -10.92 ± 2.823 | 0.7137 | 0.0083 | |
| | IF | -11.55 ± 2.609 | 0.7655 | 0.0044 | |
| | % IF | 2238 ± 505.6 | 0.7655 | 0.0044 | |

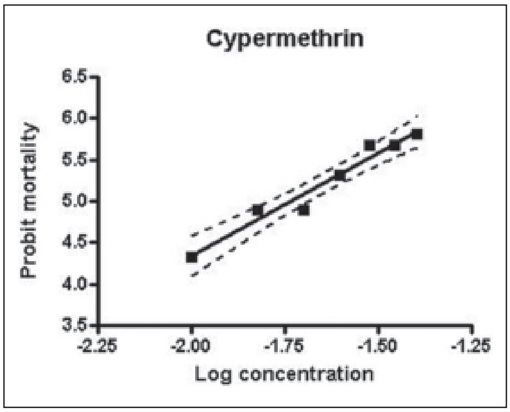


Figure 1. Dose-response graph for cypermethrin against *Rhipicephalus (Boophilus) annulatus* based on probit analysis

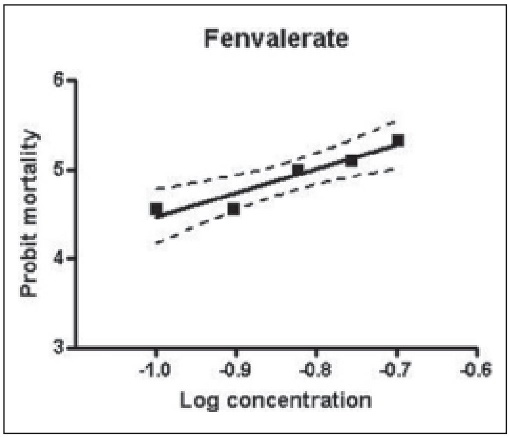


Figure 2. Dose–response graph for fenvalerate against *Rhipicephalus (Boophilus) annulatus* based on probit analysis

within 15 days of treatment is depicted in Table 4. The death of ticks was not an immediate process. Fenvalerate caused death only after 7 days while cypermethrin after 5 days of treatment. The hatching of egg laid by treated ticks was completely blocked in all groups treated at different concentrations (1000-2000 ppm for fenvalerate and 100-400 ppm for cypermetrin). We presume that this effect is due to the ability of drugs to block hatching and it is concentration independent (at the concentrations tested).

## DISCUSSION

Few chemical acaricides are available for cattle tick control (Davey & Ahrens 1984; Ware, 2000), the major compound being synthetic pyrethroids. In addition to their application as acaricides, synthetic pyrethroids are also used against agricultural pests and insects like mosquitoes (ICMR Bulletin, 2002; Ansari & Razdan, 2003; Sharma *et al.*, 2004; Tiwari *et al.*, 2010). Pyrethroids delay closing of the sodium ion channels (Vijverberg & van den Bercken, 1990), resulting in multiple nerve impulses which in turn leads to accumulation of acetylcholine (Eells *et al.*, 1992). Moreover, pyrethroids inhibit the γ- aminobutyric acid receptor causing excitability and convulsions (Ramadan *et al.*, 1988), inhibit calcium uptake by nerves (Ramadan, 1988),

Table 4. Pattern of mortality of *R. (B.) annulatus* after treatment with various dilutions of fenvalerate and cypermethrin

| Compound | Concentration (ppm) | Number of ticks | Day 1 | Day 5 | Day 7 | Day 15 | Total mortality |
|---|---|---|---|---|---|---|---|
| Fenvalerate | 1000 | 24 | 0 | 0 | 0 | 8 | 8 |
| | 1250 | 24 | 0 | 0 | 0 | 8 | 8 |
| | 1500 | 24 | 0 | 0 | 0 | 12 | 12 |
| | 1750 | 24 | 0 | 0 | 0 | 13 | 13 |
| | 2000 | 24 | 0 | 0 | 0 | 15 | 15 |
| Cypermethrin | 100 | 24 | 0 | 0 | 0 | 6 | 6 |
| | 150 | 24 | 0 | 0 | 2 | 9 | 11 |
| | 200 | 24 | 0 | 0 | 0 | 9 | 9 |
| | 250 | 24 | 0 | 0 | 2 | 15 | 17 |
| | 300 | 24 | 0 | 0 | 3 | 15 | 18 |
| | 350 | 24 | 0 | 0 | 3 | 15 | 18 |
| | 400 | 24 | 0 | 0 | 5 | 15 | 20 |

inhibit monoamine oxidase that breaks down neurotransmitters (Rao & Rao, 1993) and affect adenosine triphosphatase involved in cellular energy production, transport of metal atoms and muscle contractions (EI-Toukhy & Girgis, 1993).

Adult Immersion Test (AIT) (Drummond *et al.*, 1973) is a bioassay, which can be used to determine the relative effectiveness of acaricides in addition to diagnosis of resistance. Previously, lethal concentration of many synthetic pyrethroids was determined based on AIT. Jonsson *et al.* (2003) detected $LC_{50}$ and $LC_{99}$ values of cypermethrin against *R. (B) microplus* as 0.003g/L and 0.011g/L respectively. The $LC_{50}$ value of cypermethrin was estimated as 210 ppm in Milargo strain (Argentina) (Martins, 1996), 370 ppm against Yeerongpilly strain (Australia) (Nolan *et al.*, 1989) and 400 ppm against Porto Alegre strain (Brazil) (Martins, 1996). Jonsson *et al.* (2007) recorded $LC_{50}$ and $LC_{99}$ values of cypermethrin as 0.003% w/v and 0.00011 % w/v respectively against susceptible *R. (B) microplus* N strain, while it was 0.0003% w/v and 0.0079% w/v against susceptible Muroz starin. The $LC_{50}$ and $LC_{99}$ values of cypermethrin against *R. (B) microplus* from India were recorded as 138.5 ppm and 349.1 ppm respectively (Sharma *et al.*, 2012).

Based on available literature the $LC_{50}$ and $LC_{99}$ values of cypermethrin and fenvalerate are not available against *R. (B.) annulatus*. The present study reports the $LC_{50}$ values of synthetic pyrethroid compounds fenvalerate and cypermethrin against *R. (B.) annulatus* as 1570 ppm and 184 ppm respectively.

The ticks develop acaricidal resistance due to the indiscriminate, widespread and frequent use of chemical acaricides at incorrect/lower concentrations (FAO, 2004). Although cattle owners have reported treatment inefficiency of synthetic pyrethroids in field conditions in India, limited data on resistance of ticks to these chemicals are currently available (Kumar *et al.*, 2006, 2010; Sharma *et al.*, 2012) from the Indian subcontinent. The data developed in this study will be useful for the evaluation of resistance status of these drugs against *R. (B.) annulatus* based on adult immersion test.

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