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 LC50 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; Rajamohan, 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, fluycythrinate, 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 LC50 of field ticks and LC50 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 LC50 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):

\[
IE/IF = \frac{\text{weight of eggs laid (mg)}}{\text{weight of females (mg)}}
\]

\[
%IF = \frac{IE \text{ (control group)} - IE \text{ (treated group)}}{IE \text{ (control group)}} \times 100
\]

**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 (LC50) 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\textsubscript{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\textsubscript{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*

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Acaricide</th>
<th>Mean ticks weight per replicate ± SEM (g)</th>
<th>Mean % adult mortality within 15 days ± SEM</th>
<th>Mean eggs mass per replicate ± SEM (g)</th>
<th>Index of Fecundity ± SEM</th>
<th>Percentage Inhibition of Fecundity (%)</th>
<th>Hatching % (Visual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol (control)</td>
<td>0.9877 ± 0.010\textsuperscript{b}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>0.5098 ± 0.010\textsuperscript{c}</td>
<td>0.5199 ± 0.006\textsuperscript{b}</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2.</td>
<td>1000 ppm</td>
<td>1.1439 ± 0.068\textsuperscript{c}</td>
<td>33.33 ± 6.80\textsuperscript{b}</td>
<td>0.0726 ± 0.032\textsuperscript{b}</td>
<td>0.0502 ± 0.024\textsuperscript{b}</td>
<td>88.52</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>1250 ppm</td>
<td>0.8486 ± 0.027\textsuperscript{a}</td>
<td>33.33 ± 6.80\textsuperscript{b}</td>
<td>0.0448 ± 0.016\textsuperscript{ab}</td>
<td>0.0516 ± 0.018\textsuperscript{b}</td>
<td>89.98</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>1500 ppm</td>
<td>0.8888 ± 0.023\textsuperscript{ab}</td>
<td>39.99 ± 6.80\textsuperscript{c}</td>
<td>0.097 ± 0.007\textsuperscript{a}</td>
<td>0.0974 ± 0.007\textsuperscript{b}</td>
<td>98.55</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>1750 ppm</td>
<td>0.8257 ± 0.052\textsuperscript{a}</td>
<td>54.16 ± 7.97\textsuperscript{c}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>2000 ppm</td>
<td>0.8325 ± 0.011\textsuperscript{a}</td>
<td>62.49 ± 7.97\textsuperscript{c}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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*

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Acaricide</th>
<th>Mean ticks weight per replicate ± SEM (g)</th>
<th>Mean % adult mortality within 15 days ± SEM</th>
<th>Mean eggs mass per replicate ± SEM (g)</th>
<th>Index of Fecundity ± SEM</th>
<th>Percentage Inhibition of Fecundity (%)</th>
<th>Hatching % (Visual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Methanol (control)</td>
<td>0.9877 ± 0.010\textsuperscript{d}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>0.5098 ± 0.010\textsuperscript{d}</td>
<td>0.5159 ± 0.006\textsuperscript{e}</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2.</td>
<td>100 ppm</td>
<td>0.8062 ± 0.062\textsuperscript{ab}</td>
<td>24.99 ± 10.75\textsuperscript{a}</td>
<td>0.1878 ± 0.019\textsuperscript{a}</td>
<td>0.2337 ± 0.018\textsuperscript{a}</td>
<td>54.7</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>150 ppm</td>
<td>0.8015 ± 0.003\textsuperscript{a}</td>
<td>45.83 ± 4.16\textsuperscript{b}</td>
<td>0.0628 ± 0.023\textsuperscript{b}</td>
<td>0.0784 ± 0.029\textsuperscript{b}</td>
<td>84.8</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>200 ppm</td>
<td>0.7316 ± 0.041\textsuperscript{ab}</td>
<td>45.83 ± 14.23\textsuperscript{c}</td>
<td>0.0490 ± 0.009\textsuperscript{a}</td>
<td>0.0671 ± 0.012\textsuperscript{c}</td>
<td>86.99</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>250 ppm</td>
<td>0.8272 ± 0.030\textsuperscript{abc}</td>
<td>62.49 ± 7.97\textsuperscript{c}</td>
<td>0.0508 ± 0.025\textsuperscript{b}</td>
<td>0.0671 ± 0.029\textsuperscript{b}</td>
<td>88.54</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>300 ppm</td>
<td>0.9149 ± 0.026\textsuperscript{a}</td>
<td>74.99 ± 8.33\textsuperscript{c}</td>
<td>0.0164 ± 0.010\textsuperscript{a}</td>
<td>0.0180 ± 0.011\textsuperscript{ab}</td>
<td>96.51</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td>350 ppm</td>
<td>0.8925 ± 0.030\textsuperscript{ab}</td>
<td>74.99 ± 10.75\textsuperscript{c}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>8.</td>
<td>400 ppm</td>
<td>0.9196 ± 0.030\textsuperscript{b}</td>
<td>83.33 ± 6.80\textsuperscript{c}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>0 ± 0\textsuperscript{a}</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Variables</th>
<th>Slope ± SE</th>
<th>$R^2$</th>
<th>P value</th>
<th>LC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenvalerate</td>
<td>Mortality</td>
<td>2.675 ± 0.4909</td>
<td>0.9082</td>
<td>0.0121</td>
<td>1570 ppm</td>
</tr>
<tr>
<td></td>
<td>Egg mass</td>
<td>-2.614 ± 0.5411</td>
<td>0.8537</td>
<td>0.0085</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IF</td>
<td>-2.632 ± 0.5680</td>
<td>0.8429</td>
<td>0.0098</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% IF</td>
<td>510.0 ± 110.1</td>
<td>0.8430</td>
<td>0.0098</td>
<td></td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>Mortality</td>
<td>2.488 ± 0.2307</td>
<td>0.9588</td>
<td>0.0001</td>
<td>184 ppm</td>
</tr>
<tr>
<td></td>
<td>Egg mass</td>
<td>-10.92 ± 2.823</td>
<td>0.7137</td>
<td>0.0083</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IF</td>
<td>-11.55 ± 2.609</td>
<td>0.7655</td>
<td>0.0044</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% IF</td>
<td>2238 ± 505.6</td>
<td>0.7655</td>
<td>0.0044</td>
<td></td>
</tr>
</tbody>
</table>

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 cypermethrin). 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),
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 (El-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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