EVALUATION OF THE COMMONLY USED ACARICIDES AGAINST DIFFERENT STAGES OF THE CATTLE TICK BOOPHILUS MICROPLUS BY USING DIFFERENT IN VITRO TESTS*

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ABSTRACT
The evaluation of the efficacy of three commonly used acaricides viz. cypermethrin, deltamethrin (0.05, 0.1 and 0.2% concentrations) and amitraz (0.1, 0.2 and 0.3% concentrations) was conducted against the cattle tick Boophilus microplus. Three in vitro methods viz. Tea bag method, filter paper impregnation method and immersion method were used to evaluate the efficacy of the acaricides. Adult immersion test with discriminating doses was also done to know the resistance development if present. Comparison of different in vitro methods was done based on the efficacy against larval stages of the ticks. Among the three acaricides amitraz fared better with no indication of resistance development against B. microplus. Cypermethrin showed 30% and deltamethrin showed 10% resistance against B. microplus. Among different in vitro methods, the immersion method fared better in efficacy followed by tea bag method and filter paper method.

Key words: Boophilus microplus, Amitraz, Deltamethrin, Cypermethrin, Resistance.

INTRODUCTION
Boophilus microplus, a one host tick of cattle is one of the most widely distributed tick species and certainly the most economically important. The tick is responsible for severe losses due to tick worry, blood loss, damage to hides and injection of toxins. This tick causes significant economic losses to the livestock industry in India as a vector of Babesiosis. Chemotherapy is the most popular means of controlling these ticks.

Synthetic pyrethroids are novel among the known acaricides and by far the least toxic to mammals (Bhatia, 1974) with minimum deleterious effects on the environment. Amitraz, a formamidine compound is one of the most commonly used and rapidly acting acaricide. It has minimal toxicity to animal and no meat withholding period. Widespread and indiscriminate use of acaricidal compounds has led to the development of resistance in ticks. Early detection of resistance is essential in order to avoid further selection of resistant ticks using the same active ingredient and to delay the spread of resistance. Hence, the present study was undertaken to evaluate the efficacy of commonly used acaricides and to know the status of resistance development, if present.

MATERIALS AND METHODS
Different stages of Boophilus microplus ticks including engorged females, adult males, eggs, larvae were collected from cattle which were presented to Veterinary College, Bangalore and various Veterinary Dispensaries in and around Bangalore. In the laboratory, engorged female ticks were kept in petri dishes with a filter paper at the bottom. These petri dishes were kept in the desiccator containing saturated potassium chloride solution so as to provide a relative humidity of 80% and temperature of 25°C (Sisodia et al., 1986). The developmental stages of the tick were obtained by rearing ticks on rabbits by the ear bag method (Srivastava and Sharma, 1976).

Three acaricides were included in the study as they were commonly being used in cattle in Bangalore district. Deltamethrin and cypermethrin

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were evaluated at the concentrations of 0.05%, 0.1%, 0.2% and amitraz at the concentrations of 0.1%, 0.2%, 0.3%.

Three in vitro methods, viz. tea bag method, immersion method and filter paper impregnation methods were used in the present study. The evaluation of the efficacy of acaricide by the tea bag method as described by Gladney et al., (1972) was followed for all the stages of ticks. The efficacy in case of the filter paper impregnation method for larvae and for adult male ticks was as per Sabnis et al., (1984). The larval immersion technique as per Shaw (1966) was used in the present study. The adult immersion test with a discriminating dose (AIT-DD) for adult female ticks as recommended by FAO, (1999) with slight modifications was used to know the status of resistance.

**Tea bag method:** Tea bags (4×5.5 cm) were prepared from a heat sealable rice paper with 165 G.S.M. A known number of each developmental stages of tick such as eggs (100), larvae (50), nymphs (20), adult male ticks (10) and adult engorged females (5) were introduced separately into the tea bags which were sealed with a sealing rod. These tea bags were dipped in different concentrations of each acaricide with varying periods of time. (50 sec for eggs, 3 sec for larvae and nymphs, 40 sec for adult male ticks and 50 sec for adult engorged females)

**Filter paper impregnation method**

**a. Adult male ticks:** Whatman No. 1 filter papers of 11 cm diameter were impregnated with one ml of three different concentrations of each acaricide. One more filter paper moistened with one ml of distilled water served as control. Ten male ticks were released onto these filter papers. Then these filter papers were placed in the plastic petri dishes. Mortality was recorded after 24 hrs of exposure to acaricides and percentage mortality was calculated.

**b. Larvicidal efficacy:** Whatman No. 1 filter papers were cut into strips of 15 cm length and 2 cm width. These strips were impregnated with 1 ml of different concentrations of acaricides. They were air dried and used to line the inner surface of the test tubes of 7 cm length and 2 cm width. A batch of 50 larvae were released into each of the test tubes. The mortality was recorded at 6 hourly intervals upto 24 hrs. Percentage mortality in each group was calculated.

**Immersion method**

**a. Larval immersion method:** Fifty larvae were released on the Whatman No. 1 filter paper of 11 cm diameter in plastic petri dishes. Three milli liters of acaricide was pipetted on to the petridish lifting the filter paper. A further 4 ml was poured over the larvae and a second Whatman No. 1 filter paper of 11 cm was placed over the larvae and remaining 3 ml poured over it.

Control group was treated with 10 ml of distilled water similar to the above mentioned procedure. The immersion period was 3 min. After that the larvae were secured in separate test tubes and the assessment of mortality was made 24 hrs after immersion.

**b. Adult immersion test with discriminating doses (AIT-DD):** This was followed as per FAO, (1999) and in this test different recommended discriminating doses (DD) were used instead of different concentrations of acaricides. DD’s for amitraz, deltamethrin and cypermethrin were 2.5, 0.075 and 0.05 g/ltr respectively. Ten adult engorged females were immersed in diluted acaricides for 30 min. The ticks were dried gently on filter paper after immersion. Then these ticks were kept separately in small petridishes. The petridishes were placed in the desiccator and observed for oviposition activity for about 7 days. The percentage resistance was calculated by using the following formula

\[
\text{Resistance} \% = \left( \frac{N_t}{N_w} \right) \times 100
\]

Where \(N_t\) - Number of treated ticks laying eggs, \(N_w\) - number of untreated ticks laying eggs.

Corrected percentage mortality was calculated by Abbott’s formula as per Bagherwal et al., (1995). Efficacy of the drug was calculated on the basis of percentage of death of ticks post treatment as per Bagherwal et al., (1994).

**RESULTS AND DISCUSSION**

**Tea bag method:** Hatchability was completely inhibited at all the concentrations of cypermethrin, deltamethrin and amitraz used except 0.05% concentration of cypermethrin which showed 4% hatchability. The hatchability period of eggs exposed to 0.05% concentration of cypermethrin was 26-28 d against 20-22 d in the control group.

Complete mortality of larvae was observed in 0.2% concentration of deltamethrin and 0.3% of
amitraz. Mortality of 90%, 92% and 98% was observed at 0.05%, 0.1% and 0.2% concentrations of cypermethrin. Mortality of 94% and 98% was observed at 0.05% and 0.1 concentrations of deltamethrin. At 0.1% and 0.2% concentrations of amitraz 90% and 94% mortality was observed. In the control group mortality was not observed after 24 hours.

The acaricides were 100% effective against nymphs at the concentrations of 0.1% and above for both cypermethrin and deltamethrin, 0.2% and above for amitraz. The efficacy of 90% was observed with 0.05% cypermethrin, 0.05% deltamethrin and 0.1% amitraz concentrations. All the nymphs in the control group were alive even after 48 hours of treatment.

Observations on the efficacy of acaricides against adult male ticks revealed 100% mortality at the concentrations of 0.1% and above for both cypermethrin and deltamethrin, 0.2% and above for amitraz. Whereas 80% mortality was observed with 0.05% cypermethrin and 0.1% deltamethrin concentrations and 90% mortality was observed with deltamethrin at the 0.05% concentration. All the adult ticks of the control group were alive even after 48 hours of treatment.

Efficacy against adult engorged females was determined by oviposition activity of treated ticks and their ability to produce viable eggs. None of the treated ticks laid eggs. Thus, all the acaricides at the different concentrations used in the present study showed 100% efficacy against adult engorged female ticks. Adult engorged females in the control group laid eggs which took 20-22 days to hatch into larvae.

The efficacy of the different concentrations of acaricides on different stages of *B. microplus* by tea bag method was analysed statistically by one-way analysis of variance. Eggs, larvae, nymphs and adult male ticks showed significant difference between the different concentrations of acaricides.

**Filter paper impregnation method**

After 24 hours of exposure 100% mortality was observed in higher concentrations of acaricides under study. Mortality of 88% and 94% was observed at 0.05% and 0.1% concentrations of cypermethrin. Mortality of 92% and 94% was observed at 0.05% and 0.1% concentrations of deltamethrin. At 0.1% and 0.2% concentrations of amitraz 92% and 96% mortality was observed. In control group all larvae were alive after 24 hrs.

Mortality rate of 70%, 80% and 90% was observed at 0.05%, 0.1% and 0.2% concentrations of cypermethrin respectively. Mortality rate of 80%, 90% and 100% was observed at 0.05%, 0.1% and 0.2% concentrations of deltamethrin respectively. At 0.1%, 0.2% and 0.3% concentrations of amitraz 70%, 80% and 90% mortality was observed respectively. All the adult male ticks in the control group were alive even after 48 hours of treatment.

The efficacy of the acaricides on larvae and adult males of *B. microplus* by filter paper impregnation method was analysed statistically by one-way analysis of variance. The larvae showed significant difference whereas adult males showed no significant difference between different concentrations of the acaricides.

**Immersion method**

a. **Larval immersion method**: Complete mortality was observed with 0.2% concentration cypermethrin, 0.1% and above concentrations of deltamethrin and 0.2% and above concentrations of amitraz. Mortality of 92% and 96% was observed at 0.05% and 0.1% concentrations of cypermethrin.
TABLE 2: Comparison of three in vitro tests used for evaluating larvicidal efficacy of acaricides against *Boophilus microplus*.

<table>
<thead>
<tr>
<th>Acaricides</th>
<th>Cypermethrin</th>
<th>Deltamethrin</th>
<th>Amitraz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different in vitro tests</td>
<td>0.05%</td>
<td>0.1%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Tea bag method (% Efficacy)</td>
<td>90</td>
<td>92</td>
<td>98</td>
</tr>
<tr>
<td>Larval immersion technique (% Efficacy)</td>
<td>92</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>Filter paper method (% Efficacy)</td>
<td>88</td>
<td>94</td>
<td>100</td>
</tr>
</tbody>
</table>

Means with same superscript are not significantly different.

Mortality of 94% at 0.05% concentration of deltamethrin and 96% at 0.1% concentration of amitraz was observed. Mortality was not observed in the control group.

**a. Adult immersion test with discriminating doses (AIT-DD):** AIT-DD revealed 30% resistance to cypermethrin and 10% resistance to deltamethrin against the cattle tick *B. microplus*. No resistance was detectable with amitraz (Table. 1).

Larvicidal effect of the various concentrations of acaricides against *B. microplus* as evaluated by three different in vitro methods (Table. 2) and analysed statistically by one-way analysis of variance by using software SAS 6.12 revealed a significant difference between methods, acaricides and concentrations. The mean value of larval immersion method was higher followed by tea bag method and filter paper method. Hence immersion method gave higher efficacy than other two methods. The mean value of amitraz was higher followed by deltamethrin and cypermethrin. Hence amitraz gave better efficacy compared to other two acaricides.

The in vitro trials with deltamethrin (Butox) against *B. microplus* were conducted by Srivastava et al., (1993). Results indicated that at 0.2% concentration, deltamethrin was 100% effective. This was also observed in the present study.

Srivastava et al., (1993) evaluated the efficacy of deltamethrin *in vitro* against *B. microplus* and observed that 0.05% and higher concentrations were effective against nymphal stages of both species of ticks. This was also observed in the present study. Efficacy of deltamethrin against different stages of *H. a. anatolicum* was evaluated *in vitro* by Bagherwal et al., (1994). They used three different concentrations i.e. 25, 50, 75 ppm and found 81.3, 96.3 and 98.2% efficacy respectively. These results are different from that of the present study wherein the immersion method was used where exposure time was more compared to tea bag method and different species of tick.

Efficacy of deltamethrin (Butox) against different stages of *B. microplus* was evaluated *in vitro* by Srivastava et al., (1993). Adults of both the ticks showed cent percent mortality at 0.2% and higher concentration. These results are in agreement with the present study.

Acaricidal efficacy on the biological activities of female *B. microplus* against butox (deltamethrin) was evaluated by Stuti Vatsya and Das, (2004). At the concentrations of 0.005% and 0.05% deltamethrin inhibited egg production in ticks which is in accordance with the present study.

AIT-DD conducted on *B. microplus* engorged female ticks revealed only susceptible population of ticks. The results of all the trials were similar, with all treated ticks shriveling and immediately succumbing to the effect of both deltamethrin and flumethrin (Shailaja, 2006). However in the present study deltamethrin showed 10% resistance against *B. microplus*. This observation has been made in Karnataka state for the first time while the absence of resistance has been reported found in ticks of Tamil Nadu (Shailaja, 2006). The possibility of overuse of deltamethrin in Karnataka is to be examined with relation to the indication of resistance development.
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REFERENCES


