In vitro evaluation of acaricidal activity of fipronil against Haemaphysalis bispinosa based on adult immersion test

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Abstract. Fipronil is a phenylpyrazole family insecticide which mainly affects the nervous system of insects. In the present study, the in vitro acaricidal effects of the compound against the widely prevalent multihost tick, Haemaphysalis bispinosa was assessed. The lowest concentration at which complete adult tick mortality was observed was at 25 ppm while complete absence of egg mass observed at 10 ppm. Hundred per cent inhibition of fecundity was observed at 1 ppm while complete blocking of hatching of the laid ova was observed even at 500 ppb.

INTRODUCTION

Control of tick infestations and the transmission of tick-borne diseases remain a challenge for the cattle industry in tropical and subtropical areas of the world (Lodos et al., 2000). The global loss due to ticks and tick-borne diseases (TTBDs) in cattle range between US $ 13.9 and 18.7 billion annually (De Castro, 1997). In India, the cost of TTBD control in animals has been estimated to be US $ 498.7 million per annum (Minjauw & Mc Leod, 2003). Chemical control with acaricides was considered as one of the best methods, but it was shown recently that ticks have developed resistance against a range of acaricides (Martins et al., 1995). Hence, for effective pest control around the world it is necessary to have available a range of compounds with different modes of action to enable the rotation of these chemicals and so help to manage existing resistance (Graf et al., 2004).

Fipronil, a compound of N-phenylpyrazole with a trifluoromethylsulfinyl substituent, acting at the gamma-aminobutyric acid (GABA) receptor to block the chloride channel, is considered more toxic to the insects than to the vertebrates (Hainzl et al., 1998). Fipronil is available for control of ticks in several countries in Latin America, but has not been registered in the US and some other countries for use on food animals (George et al., 2004). Previously, the compound was tested in vitro for acaricidal effects against Rhipicephalus (Boophilus) microplus (Davey et al., 1999) of cattle and Dermacentor reticulatus (Bonneau et al., 2011) of dogs. But there are no published reports on efficacy of the compound against Haemaphysalis bispinosa which is the widely prevalent multihost tick species in
South India (Geevarghese et al., 1997; Prakasan & Ramani, 2007).

Hence, the present investigation focuses on the in vitro effect of fipronil against *H. bispinosa*.

**MATERIALS AND METHODS**

**Fipronil**

Fipronil (10mg) pure compound (AccuStandard®, Inc., USA) was dissolved in 10 ml of acetone and then further diluted to make different concentrations in water viz., 500 ppb, 1 ppm, 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm.

**Ticks**

Fully engorged adult *H. bispinosa* female ticks collected from infested calves were washed with distilled water and dried using clean soft tissue paper.

**Experimental protocol**

Adult immersion test (AIT) was performed as per the protocol described by Drummond et al. (1973). Four replicates of six ticks each were used for testing of single dilution of fipronil. Six ticks were immersed in the solution (10 ml) at room temperature for two minutes in a 50 ml beaker with gentle agitation. One per cent acetone was used as control. Ticks were recovered from the solutions, dried and placed in a plastic specimen tube (25 X 50 mm). They were incubated at 28°C and 80 per cent relative humidity in a BOD incubator.

**Per cent adult mortality, inhibition of fecundity, hatching**

Adult tick mortality was observed up to 19th day after immersion. After oviposition, the eggs laid by the female ticks were collected and weighed. The index of egg laying (IE) and percentage inhibition of fecundity (IF) were calculated (FAO, 2004) as follows:

- Index of egg laying (IE) = weight of eggs laid (g)/weight of females (g)
- Percentage inhibition of fecundity (IF) = [(IE control group - IE treated) X 100]/IE control group.

Hatching percentage of eggs was calculated visually.

**Statistical analysis**

All the data were expressed as the mean ± SEM. Groups were compared using one-way ANOVA for repeated measurements using SPSS software. Duncan’s test was used for post-hoc analysis. A value of P<0.05 was considered significant.

**RESULTS**

Results (Table 1) of adult immersion test (AIT) with *H. bispinosa* revealed that per cent mortality of 50 and inhibition of fecundity of 79.9 were observed at the lowest concentration (500 ppb) tested. From 25 ppm onwards, the mortality, inhibition of fecundity and blocking of hatching of laid ova were 100 per cent. At 1 ppm and 10 ppm, 62.5 and 91.6

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Fipronil concentration</th>
<th>Mean ticks weight ± SEM</th>
<th>Mean % adult mortality within 15 days ± SEM</th>
<th>Mean eggs mass ± SEM</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>500 ppb</td>
<td>0.676 ± 0.023ª</td>
<td>49.995 ± 9.621ª</td>
<td>0.069 ± 0.044ª</td>
<td>0.100 ± 0.063ª</td>
<td>79.9</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>1 ppm</td>
<td>0.672 ± 0.016ª</td>
<td>62.497 ± 12.499ª</td>
<td>0.064 ± 0.006ª</td>
<td>0.096 ± 0.011ª</td>
<td>80.81</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>10 ppm</td>
<td>0.651 ± 0.020ª</td>
<td>91.665 ± 8.335ª</td>
<td>0 ± 0ª</td>
<td>0 ± 0ª</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>25 ppm</td>
<td>0.595 ± 0.053</td>
<td>100ª</td>
<td>0 ± 0ª</td>
<td>0 ± 0ª</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>50 ppm</td>
<td>0.671 ± 0.011ª</td>
<td>100ª</td>
<td>0 ± 0ª</td>
<td>0 ± 0ª</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>75 ppm</td>
<td>0.711 ± 0.031ª</td>
<td>100ª</td>
<td>0 ± 0ª</td>
<td>0 ± 0ª</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td>100 ppm</td>
<td>0.664 ± 0.028ª</td>
<td>100ª</td>
<td>0 ± 0ª</td>
<td>0 ± 0ª</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>8.</td>
<td>Control (1% Acetone)</td>
<td>0.584 ± 0.022</td>
<td>0 ± 0ª</td>
<td>0.296 ± 0.052</td>
<td>0.499 ± 0.075</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

n = 4, Values are Mean ± SEM, means bearing superscript ‘a’ indicate significant difference (P<0.05), when compared with the control.
percent adult tick mortality were observed. Egg laying by treated ticks was completely blocked from concentrations of 10 ppm onwards.

DISCUSSION

Fipronil is an outstanding new insecticide for crop protection with good selectivity between insects and mammals. Fipronil at low dosage provides long term protection against lepidopterous and orthopterous pests on crops and coleopterous larvae in soil (Hainzl et al., 1998). Fipronil binds to three types of calcium-channels on the membranes of neurons of insects, preventing calcium ion influx into the cell. One of these types of channels is mediated by the neurotransmitter gamma-aminobutyric acid (GABA) and the other two are mediated by glutamate (FAO, 2009). GABA is strictly an inhibitory neurotransmitter – when GABA is activated at a synapse, the nerve impulse stops.

Fipronil affects the nervous system of insects and has both contact and ingestion activity (FAO, 2009). Fipronil exhibits differential binding affinity for GABA\textsubscript{A} receptor subunits, with a higher binding affinity for insect receptor complexes compared to mammalian complexes. The lower binding affinity for mammalian receptors enhances selectivity for insects and increases the margin of safety for people and animals (Jackson et al., 2009). Differences in GABA receptor sensitivity, assayed by displacement of 4'-ethynyl-4-n-(2, 3-3H\textsubscript{2}) propylbicycloorthobenzoate ((3H) EBOB) from the noncompetitive blocker site, appear to be a major factor in fipronil being much more toxic to the insects (house fly and fruit fly) than to the vertebrates (humans, dogs, mice, chickens, quail and salmon) (Hainzl et al., 1998).

There are only few reports on the use of fipronil for the control of ticks. Davey et al. (1998), reported that fipronil applied as pour-on to cattle infested with *R. (B.) microplus* and confined in an open–sided barn, had therapeutic efficacy greater than 90 per cent and a similar degree of persistent protection against larval reinfestation for eight weeks after treatment. Later, Davey et al. (1999), found that a single treatment had no effect on the tick population and two to four treatments at various intervals were required for significant reduction of the tick population. Under field conditions, persistent efficacy of a single pour-on treatment of fipronil on cattle was only for two to three weeks (Davey et al., 1999). Bonneau et al. (2011) studied the efficacy of a fipronil based spot-on (Effipro\textsuperscript{D}, Virbac) on dogs experimentally infested with tick *Dermacentor reticulatus* and concluded that those ticks that attached to the drug treated animal could be killed within 24 - 48 hours.

Hence, it can be concluded that fipronil is a highly effective, broad spectrum insecticide with potential for control of ticks. The major advantage observed was that even at very low concentration (10 ppm), the in vitro application of fipronil prevented the egg laying, thereby prevented development of future generations from the treated *H. bispinosa* ticks.

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REFERENCES


