Determination of LC₅₀ and LC₉₅ values of fipronil against *Haemaphysalis bispinosa* based on adult immersion test

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Received: 11 July 2012 / Accepted: 13 September 2012 / Published online: 27 September 2012
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Abstract The pattern of mortality of *Haemaphysalis bispinosa* ticks against fipronil was studied based on adult immersion test. Mortality occurred during 10–19 days post-treatment. The LC₅₀ and LC₉₅ values were calculated as 0.53 and 7.045 ppm, respectively.

Keywords Fipronil · Acaricidal activity · Mortality · LC₅₀ · LC₉₅

Ticks cause great economic losses to livestock in the world and have adverse effect on livestock host in several ways (Snelson 1975). In India alone, the cost of tick and tick borne diseases (TTBDs) in animals has been estimated to the tune of US\$ 498.7 million (approx. 2,000 crores) per annum (Ghosh et al. 2007a). The predominantly used acaricides for controlling TTBDs are organophosphates, carbamates, pyrethroids, BHC/cyclodienes, amidines, macrocyclic lactones, benzylphenylureas, avermectins and organochlorines (Ghosh et al. 2007a).

The progressive evolution of resistance of ticks affecting cattle to almost all of the available acaricides has frustrated the efforts in cattle producers to manage ticks and tick borne diseases (George et al. 2004). Early detection of the resistance, subsequent withdrawal and replacement with another acaricide would sustain the susceptible tick population in the field (Ghosh et al. 2007a).

Fipronil is the first phenyl pyrazole compound registered for use to control pest (Hainzl and Casida 1996; Hatton et al. 1988), which block γ -aminobutyric acid (GABA) receptors and interferes with the passage of chloride ions through a GABA-gated channel. At low concentrations, it disrupts nervous system function and at higher doses causes death (Cole et al. 1993).

Out of the 166 valid *Haemaphysalis* tick species of the world, only 44 species (Ghosh et al. 2007b) were reported from different parts of India. Among the *Haemaphysalis* species reported from Kerala and Tamilnadu, the most common species is *Haemaphysalis bispinosa* (Latha et al. 2004; Prakasan and Ramani 2007). The present communication deals with the in vitro acaricidal effect of fipronil and focuses on the determination of LC₅₀ and LC₉₅ values of the drug against *H. bispinosa*.

Fipronil pure compound was obtained from AccuStandard, U.S.A. An amount of 10 mg of fipronil compound was dissolved in 10 ml of acetone, to make 1,000 ppm solution. From the stock solution, working dilutions were made in distilled water, so that 500 ppb, 1, 10, 25, 50, 75 and 100 ppm concentrations were achieved.

Fully engorged adult *H. bispinosa* collected from infested animals, were washed with water and mopped dry using tissue paper. Adult immersion test was performed based on Drummond et al. 1973. Four replicates each with six numbers of ticks were used for each concentration of the compound. Group of six numbers of ticks were

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weighed prior to the experiment and they were immersed for 2 min in the respective dilution (10 ml) in a 50 ml beaker. One per cent acetone was used as control. Ticks were recovered from the solution, mopped dry using tissue paper towel and placed in separate plastic specimen tube (25 × 50 mm). These tubes were incubated at 28 °C and 80 % relative humidity in a BOD incubator.

The number of adult tick died on days 1, 5, 10 and 19 post treatment was determined. Dose response data were analyzed by probit method (Finney 1952). The LC_{50} and LC_{95} values of fipronil against *H. bispinosa* were determined by applying regression equation analysis to the probit transformed data of mortality.

Out of the seven concentrations (0.5–100 ppm) tested, 100 % mortality was observed at concentrations above 25 ppm (Table 1). No immediate mortality after application of fipronil was observed. However mortality was observed from 5 days after treatment. Majority of death occurred mainly between 10th and 19th day post-treatment.

From the graph (Fig. 1), the linear regression equation obtained was

$$Y = 1.4908x + 5.3759,$$

$$R^2 = 0.9375$$

$$LC_{50} = 0.53 \text{ ppm and } LC_{95} = 7.045 \text{ ppm}$$

Table 1 Pattern of mortality of *H. bispinosa* after treatment with various dilutions of fipronil

Fipronil (µg/ml)	No. of ticks	1st Day	5th Day	10th Day	19th Day	Total mortality
0.5	24	0	0	1	11	12
1	24	0	0	3	14	17
10	24	0	2	5	14	21
25	24	0	3	7	14	24
50	24	0	2	5	17	24
75	24	0	5	7	12	24
100	24	0	5	9	10	24

Fig. 1 Graph showing log concentrations of fipronil on X-axis and probit mortality calculated from Finney's table on Y-axis

Fipronil is systemic insecticide acting agonistically on the GABA gated chloride channel in target pests (Brown 2005; FAO 2009). Fipronil was used previously as a control agent for moths and grasshoppers on crops and beetle larvae in soils (Hainzl and Casida 1996). 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 GABA and the other two are mediated by glutamate (FAO 2009). Fipronil has excellent therapeutic and persistent activity against fleas on both cats and dogs (Blagburn et al. 1994; Postal et al. 1995) and *Boophilus microplus* ticks (Hunter et al. 1994).

In the present study, the LC_{50} and LC_{95} values of fipronil against the *H. bispinosa* were determined as 0.53 and 7.045 ppm, respectively based on AIT. Previously, Oliveira et al. 2008 reported LC_{50} of 10 ppm against *Rhipicephalus sanguineus*. Also, LC_{50} and $LC_{99.9}$ values of fipronil against susceptible strain of *Rhipicephalus (Boophilus) microplus* were determined as 0.75 and 2.49 ppm, respectively using AIT (Castro-Janer et al. 2009). Hence, LC_{50} and LC_{95} values of the drug against *H. bispinosa* are similar to that of *R. (B.) microplus*.

Adult immersion test is more reliable for diagnosis of resistance against fipronil compared to other tests like larval packet test (Castro-Janer et al. 2009). Resistance of *R. (B.) microplus* against fipronil was already reported in Brazil (Castro-Janer et al. 2010) and Uruguay (Cuore et al. 2007; Castro-Janer et al. 2009) where the drug was used for control of ticks in livestock. Hence, the data developed in the present study will be useful for evaluation of resistance status of the drug based on AIT against tick species of bovines of Indian subcontinent, since the drug has been recently introduced into the Indian market.

Acknowledgments Financial support from Indian Council of Agricultural Research for the National Agricultural Innovation Project entitled “Study of herbal acaricides as means to overcome the development of resistance in ticks to conventional acaricides” is thankfully acknowledged.

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