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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 contries 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 28ºC 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 1. Acaricidal effects of *in vitro* application of fipronil *against H. bispinosa* based on AIT

Sl. No	Fipronil concentration	Mean ticks weight ± SEM	Mean % adult mortality within 15 days ± SEM	Mean eggs mass ± SEM	Index of fecundity ± SEM	Percentage Inhibition of Fecundity (%)	Hatching % (Visual)
1.	500 ppb	0.676 ± 0.023^a	49.995 ± 9.621^a	0.069 ± 0.044^a	0.100 ± 0.063^a	79.9	0
2.	1 ppm	0.672 ± 0.016^a	62.497 ± 12.499^a	0.064 ± 0.006^a	0.096 ± 0.011^a	80.81	0
3.	10 ppm	0.651 ± 0.026^a	91.665 ± 8.335^a	0 ± 0^a	0 ± 0^a	100	0
4.	25 ppm	0.595 ± 0.053	100^a	0 ± 0^a	0 ± 0^a	100	0
5.	50 ppm	0.671 ± 0.011^a	100^a	0 ± 0^a	0 ± 0^a	100	0
6.	75 ppm	0.711 ± 0.031^a	100^a	0 ± 0^a	0 ± 0^a	100	0
7	100 ppm	0.664 ± 0.028^a	100^a	0 ± 0^a	0 ± 0^a	100	0
8	Control (1% Acetone)	0.584 ± 0.022	0 ± 0	0.296 ± 0.052	0.499 ± 0.075	0	100

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 $GABA_A$ 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, 3-3H2) 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^R, 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

Bonneau, S., Gupta, S., Maynard, L. & Eun, H. (2011). Antiparasitic efficacy of a new fipronil-based spot-on formulation on dogs experimentally infested with ticks *Dermacentor reticulatus*. *International Journal of Applied Research and Veterinary Medicine* **9**: 1-5.

Davey, R.B., Ahrens, E.H., George, J.E., Hunter, J.E. & Jeannin, P. (1998). Therapeutic and persistent efficacy of fipronil against *Boophilus microplus* (Acari: Ixodidae) on cattle. *Veterinary Parasitology* **74**: 261-276.

Davey, R.B., George, J.E. & Hunter, J.S. (1999). Evaluation of a pour-on formulation of fipronil against *Boophilus annulatus* (Acari: Ixodidae) under natural South Texas field conditions. *Experimental and Applied Acarology* **23**: 351-364.

De Castro, J.J. (1997). Sustainable tick and tick–borne diseases control in live stock improvement in developing countries. *Veterinary Parasitology* **71**: 77-97.

Drummond, R.O., Ernst, S.E., Trevino, J.L., Gladney, W.J. & Graham, O.H. (1973). *Boophilus annulatus* and *Boophilus microplus*: laboratory tests for insecticides. *Journal of Economic Entomology* **66**: 130-133.

FAO (2009). Specifications and evalutions for fipronil. http://www.fao.org/agriculture/crops/core-hemes/theme/pests/pm/jmps/en/ pp 1-54.

Geevarghese, G., Fernandes, S. & Kulkarni, S.M. (1997). A checklist of Indian ticks (Acari: Ixodoidea). *Indian Journal of Animal Sciences* **67**: 566-574.

George, J.E., Pound, J.M. & Davey, R.B. (2004). Chemical control of ticks on cattle and the resistance of these parasites to acaricides. *Parasitology* **129**: S353-366.

Graf, J.F., Gogolewski, R., Leach-bing, N., Sabatini, G.A., Molento, M.B., Bordin, E.L. & Arantes, G.J. (2004). Tick control: an industry point of view. *Parasitology* **129**: S427-S442.

Hainzl, D., Cole, L.M. & Casida, J.E. (1998). Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chemical Research in Toxicology* **11**: 1529-1535.

Jackson, D., Cornell, C.B., Luukinen, B., Buhl, K. & Stone, D. (2009). Fipronil Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. http://npic.orst.edu/factsheets/fiptech.pdf (Accessed during March 2011).

Lodos, J., Boue, O. & de la Fuente, J.A. (2000). Model to simulate the effect of vaccination against *Boophilus* ticks on cattle. *Veterinary Parasitology* **87**: 315-326.

Martins, J.R., Corrêa, B.L., Ceresér, V.H. & Arteche, C.C.P. (1995). A situation report on resistance to acaricides by the cattle tick *Boophilus microplus* in the state of Rio Grande do Sul, Southern Brazil. *In*: Rodriguez, C.S., Fragoso, S.H. (Eds.), Resistencia y Control en Garrapatas y Moscas de Importancia Veterinaria III. *Seminario Internacional de Parasitologia Animal*. Acapulco, Mexico, pp.1-8.

Minjauw, B. & Mc Leod, A. (2003). Tick – borne diseases and poverty: the impact of ticks and tick- borne diseases on the livelihood of small scale and marginal livestock owners in India and eastern and southern Africa. Research report DFID Animal Health Programme. Centre for Tropical Veterinary Medicine, University of Edinburgh, UK.

Prakasan, K. & Ramani, N. (2007). Tick parasites of domestic animals of Kerala, South India. *Asian Journal of Animal and Veterinary Advances* **2**: 74-80.