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Detection of deltamethrin resistance in eggs and larva of *Rhipicephalus (Boophilus) microplus*

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ABSTRACT

Rhipicephalus (Boophilus) microplus is the most common tick species in India infesting cattle and buffaloes and cause significant economic losses to dairy and leather industries by adversely affecting the milk production and quality of hides. Egg hatch assay and larval packet test were used to evaluate the comparative acaricidal efficacy of the market formulations of deltamethrin, cypermethrin and amitraz on eggs and larval stages of *R. (B.) microplus*. The regression graphs of probit hatchability, % inhibition of hatching (IH) of eggs and probit mortality were plotted against log concentrations of various acaricides. The results revealed that cypermethrin and amitraz were effective and produced complete inhibition of hatching. LC_{95} values of cypermethrin and amitraz calculated by larval packet test were estimated to be 244.91 ppm and 369.83 ppm, respectively. On the other hand, deltamethrin failed to completely inhibit the hatching. LC_{95} values by egg hatch assay and larval packet test were estimated to be 42.27 ppm and 39.36 ppm, respectively. The data on field status of acaricide resistance from the area with diversified animal genetic resources will be helpful to adopt suitable strategy to overcome the process of development of resistance in ticks.

Key words: Acaricides, Cattle tick, Egg hatch assay, Larval packet test, Resistance

The cattle tick, *Rhipicephalus (Boophilus) microplus* is widely distributed and has great impact on cattle breeding in the tropical and subtropical world, particularly in India due to macroclimatic factors which play a major role on the seasonal population dynamics (Singh *et al.* 2000). This is aggravated by the increasing numbers of acaricide-resistant tick populations. As per Crow (1957), resistance is a hereditary characteristic, defined as a change in the genetic composition of a defined population in response to selection. At present, tick control in India is based on large scale repeated use of synthetic acaricides of 3 major classes, namely organophosphates, pyrethroids and carbamates (Shyma *et al.* 2014). Several non-organophosphate (OP) classes of pesticides are effective against arthropod pests, environmentally safe and relatively less toxic to mammals and other non-target organisms when compared to OP compounds. Among these pesticides, the synthetic pyrethroids; deltamethrin and cypermethrin are commercially available in India, and presently the predominant acaricides used to control tick in the country (Mathivathani *et al.* 2011, Sharma *et al.* 2012). The formamidine (amitraz) was initially introduced to control organophosphate (OP) resistant ticks, simultaneously when the synthetic pyrethroids (SP) were introduced, but owing to its higher cost, its use was initially limited (Haque *et al.* 2014). The first widespread report covering 27 areas located in 6 agro-climatic zones against organophosphate and synthetic pyrethroids in *R. (B.) microplus* was reported by Kumar *et al.* (2011), Shyma *et al.* (2013) and Sharma *et al.* (2012). But Gujarat plains and hills agro-climatic zone of the sub-continent was not explored. However, North Gujarat is predominated with the dairy farms comprising mainly of crossbred cattle population. Report of Singh *et al.* (2015) is the only available information regarding resistance status of *R. (B.) microplus* tick isolates from this agro-climatic zone. The present study was aimed for estimation of comparative efficacy of market formulations of acaricides (deltamethrin, cypermethrin and amitraz) on the eggs and larval stages of *R. (B.) microplus* ticks.

MATERIALS AND METHODS

Collection of samples: Live engorged adult female *R. (B.) microplus* ticks were collected from sheds of dairy animals comprising crossbred cattle as well as buffaloes of varying age and from the vicinity of their pens, from Banaskantha district of Gujarat state, using simple stratified random sampling procedure. Data were collected from livestock keepers related to frequency, type, and mode of acaricide treatment adopted by them and their experiences

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about the commonly used acaricides. The ticks were collected in glass vials, brought to the laboratory, cleaned, labelled, and kept at 28°C and 75–85% relative humidity in labelled glass bottle with the mouth covered by muslin cloth for oviposition.

Acaricides: Farmers of the areas under study have reported frequent applications of chemical acaricides in particular deltamethrin, cypermethrin and recently amitraz, without maintaining an optimum concentration for the control of ticks mainly due to low efficacy of most of the marketed products. The commercially available preparations of cypermethrin (Ectomin, 10%), deltamethrin (Butox, 1.25%) and amitraz (Extick, 12.5%) were procured from the local market. For the experimental bioassay, various concentrations of cypermethrin (100, 200, 400, 800 and 1600 ppm), deltamethrin (12.5, 25, 50, 100 and 200 ppm), and amitraz (125, 250, 500, 1000 and 2000 ppm) were prepared in distilled water from the stock solution and tested against the eggs and larvae of *R. (B.) microplus*.

Egg hatch assay (EHA): With slight modifications, EHA was conducted as per Ribeiro *et al.* (2008). In 1 ml of the test solution, approximately 100 *R. (B.) microplus* embroynated eggs were immersed in a glass tube for 5 min. Subsequently, the solution was decanted and after evaporation of the solvent, the tube was covered with a muslin cloth. Eggs were incubated at 28°C and 75–85% relative humidity for 14 days, until incubation period was over. Each treatment comprised 3 replicates and water was used as control. The results were compared based on the parameters estimated as given:

Hatching (%) = (Number of larvae hatched)/(Total number of incubated eggs)

$$\text{Percentage inhibition of hatching (\% IH)} = \frac{\text{(Hatching \% control - Hatching \% treated)}}{\text{(Hatching \% control)}} \times 100$$

Larval packet test (LPT): Six-hundred microliter of different concentrations of cypemethrin, deltamethrin, and amitraz diluted in distilled water were used to impregnate 3.75 cm × 8.5 cm filter paper rectangles for conducting larval packet test (LPT) (FAO 1971). Treated packets containing approximately 150 live larvae were incubated for 24 h in BOD incubator at 28°C, 80 to 90% RH. Three replicates of each concentration and two distilled water controls were used. After incubation, packets were checked for mortality of larvae. Mortality per cent was calculated.

Statistical analysis: Dose response data were analyzed by probit method (Finney 1952) using GraphPad Prism 4 software. LC_{95} values (concentrations causing 95% inhibition of hatching) of various acaricides were determined by applying regression equation analysis to the data of inhibition of hatching (%). Regression curves of larval mortality data were also plotted against values of acaricide concentrations by log dosage probit mortality analysis (Finney 1971) for determination of LC_{95} values of cypermethrin, deltamethrin and amitraz for *R. (B.) microplus* tick isolates. Discriminating doses for all acaricides were determined both for LPT and EHA as $2 \times LC_{95}$ (Jonsson *et al.* 2007).

RESULTS AND DISCUSSION

Areas with report of high incidence of tick infestation were selected in the present study. Veterinarians of the locality often complain about treatment failure of commonly applied acaricides in tick infestation case. Effect of acaricides on egg stage and larval stage of ticks was undertaken and the results of egg hatch assay revealed that all the concentrations of cypermethrin and amitraz caused complete inhibition of hatching and hence recorded zero hatching percentage. However, deltamethrin failed to completely inhibit the hatching. The ratio of number of hatched larvae to total number of incubated eggs gives an idea of hatching percentage. This ratio integrates both mortality of eggs and the inability of viable eggs to hatch. Hatchability as low as 1.3% was recorded at the highest concentrations of deltamethrin. The slope of IH% (95% CL), Y intercept (95% CL), value of goodness of fitness (R^2), P values, LC_{95} and DD of deltamethrin used against the eggs of *R. (B.) microplus* are shown in Table 1. Dose dependent probit IH curve of deltamethrin against eggs of *R. (B.) microplus* is shown in Fig. 1. Recently, Haque *et al.* (2014)

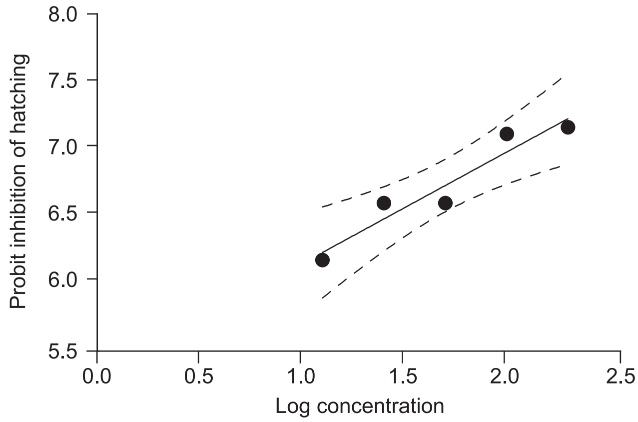

Fig. 1. Dose-inhibition of hatching (probit) curve of deltamethrin against eggs of *R. (B.) microplus*.

Table 1. Dose dependent probit inhibition of hatching of acaricides to *R. (B.) microplus*

Acaricide	Slope (95% CL)	*Y*-intercept (95% CL)	R^2	*P* value	LC_{95} (ppm)	DD (ppm)
Deltamethrin	0.84± 0.1461 0.37 to 1.30	5.28± 0.26 4.47 to 6.09	0.9163	0.0106[b]	42.27	84.54
Cypermethrin	-	-	-	-	NC	NC
Amitraz	-	-	-	-	NC	NC

[NC]Not calculated.

Table 2. Dose dependent probit mortality of different acaricides to *R. (B.) microplus* larva

Acaricide	Slope (95% CL)	*Y*-intercept (95% CL)	R^2	*P* value	LC_{95} (ppm) (95% CL)	DD (ppm) (95% CL)
Deltamethrin	1.74±0.24 0.973 to 2.51	3.86±0.42 2.52 to 5.21	0.9455	0.0055[a]	39.36	78.72
Cypermethrin	0.74±0.10 0.426 to 1.055	4.87 ± 0.26 4.04 to 5.70	0.9493	0.0049[a]	244.91	489.82
Amitraz	1.23±0.45 -0.22 to 2.67	3.49±1.24 -0.46 to 7.44	0.7084	0.0738[b]	369.83	739.66

[a] $P < 0.01$, [b] $P < 0.1$.

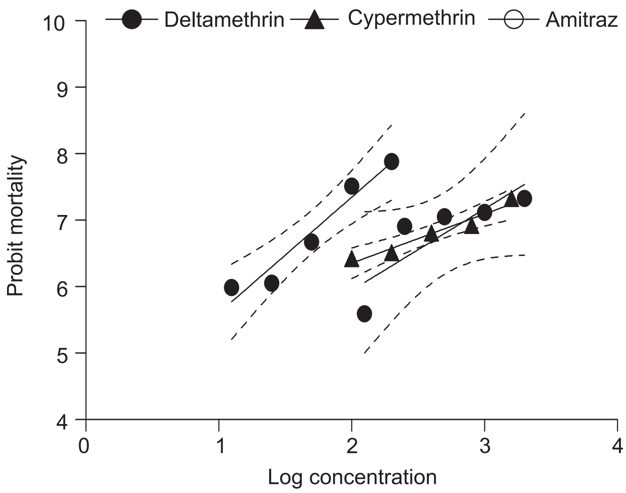

Fig. 2. Dose-probit mortality curve of acaricides against larvae of *R. (B.) microplus*.

studied the effect of commonly used market formulated acaricides (amitraz, cypermethrin, deltamethrin, fenvalerate and flumethrin) on the eggs of *R. (B.) microplus*, using EHA and concluded that cypermethrin and deltamethrin were found ineffective with a hatchability of 31.0±6.1, 40.0±5.2 and 19.3±1.7% recorded at the highest concentration of cypermethrin, deltamethrin and fenvalerate, stating that much higher concentration would be required for the efficient control of the egg stage whereas flumethrin and amitraz showed complete inhibition of hatching.

The cattle tick *R. (B.) microplus,* is widely distributed throughout tropical and subtropical regions of the world and affects dairy animals in North Gujarat (Ghosh *et al.* 2006). Larval packet test was simultaneously conducted and showed comparable result against the acaricides. The regression graphs of larval probit mortality of *R. (B.) microplus* was plotted against log values of progressively increasing concentrations of both the pyrethroids along with amitraz (Fig. 2). The slope of dose dependent mortality (95% CL), Y intercept (95% CL), value of goodness of fitness (R^2), P values, LC_{95} and DD of deltamethrin, cypermethrin and amitraz used against the larvae of *R. (B.) microplus* are presented in Table 2. LC_{95} values of deltamethrin, cypermethrin and amitraz was estimated to be 39.36, 244.91 and 369.83 ppm, respectively. The coefficients of determination (R^2 values) of estimations were more than 90% both for deltamethrin and cypermethrin, indicating a good fitting of the data in the probit model (Table 2). However, R^2 value of curve fitted for amitraz was only 70%.

The problem of ticks and tick-borne diseases is particularly relevant in India because of the congenial environmental conditions for tick survival throughout the year and maintenance of susceptible crossbred animals to improve the production of milk and other animal products (Singh *et al.* 2000, Shyma *et al.* 2012). The work was undertaken based on the reports of treatment inefficiencies of the commonly applied acaricides in the region and people were looking for alternate to the chemical acaricides (Shyma *et al.* 2014). Use of deltamethrin was predominated and cypermethrin and amitraz were introduced comparatively very late.

As per Sharma *et al.* (2012), the LC_{95} values of deltamethrin and cypermethrin by adult immersion test against susceptible IVRI-I line of *R. (B.) microplus* were reported as 29.6 and 349.1 ppm, respectively. Similarly, in larval packet test, the LC_{95} values of deltamethrin and cypermethrin were 35.5 ppm and 350.7 ppm, respectively, against larvae of susceptible IVRI-I line of *R. (B.) microplus*. However, the results of current study demonstrate that much higher concentration of deltamethrin would be required for the efficient control of the eggs as well larval stages, thus indicating that the dose at which the deltamethrin is being used in field conditions is becoming less effective and there is need of revalidation. Cypermethrin was effective against *R. (B.) microplus*, which is duly supported by the results of EHA.

Kumar *et al.* (2014) in his experiments with susceptible IVRI-I line of adult *R. (B.) microplus* determined LC_{95} of 487.7 ppm for amitraz. Results of this study when compared with the susceptible line indicated that the field strain is susceptible to amitraz. The finding is further supported by the results of EHA. Recently, Singh *et al.* (2015) has reported high level of resistance with resistance factor (RF) of 24.78 in adult *R. (B.) microplus*. However, RF was calculated based on commercially recommended dosage of amitraz.

On the basis of obtained data, an indication can be drawn that there is problem of emerging resistance in *Rhipicephalus (B.) microplus* to chemical acaricides which are recently being used heavily in the region and there should be an alert on good practices aiming for tick control required to be recommended in order to monitor resistance and judicious use of acaricides.

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