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Original article

A comparative study on cypermethrin resistance in *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* from Punjab (India)

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ABSTRACT

A study to evaluate cypermethrin resistance in *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Muktsar and Mansa districts of Punjab state, India, was conducted by using adult immersion test (AIT). The regression graphs of probit mortality of ticks plotted against log values of concentrations of cypermethrin was utilized for the determination of slope of mortality, lethal concentration for 50% (LC_{50}), and the resistance factor (RF). On the basis of the data generated on variables (mortality, egg mass weight, reproductive index, and percentage inhibition of oviposition), the resistance levels were categorized. Resistance to cypermethrin was categorized as level II and I in *R. (B.) microplus* collected from Muktsar and Mansa districts, respectively, whereas, *H. anatolicum* from both locations showed a susceptible status. The RF values of Muktsar and Mansa field samples of engorged *R. (B.) microplus* (5.48 and 2.18, respectively) were much higher as those of engorged *H. anatolicum* (1.12 and 0.82, respectively) indicating a lower level and slower rate of development of cypermethrin resistance in multi-host ticks. The data generated in the current study might be of immense help in formulating suitable control measures against ticks and tick-borne diseases of animals.

Introduction

Ticks and tick-borne diseases are a major problem to livestock health worldwide. Their significance depends on the region, the species involved, the host population(s) involved, and socioeconomic and technological advances in control measures (Solis, 1991). Losses attributable to ticks are caused either directly by tick bites (Biswas, 2003; Jongejan and Uilenberg, 2004), blood loss, damage to hides and udders, decreased milk yield (Sutherst, 1983; Sajid et al., 2007), or indirectly through mortality or morbidity caused by the pathogens transmitted by or associated with the ticks. The global economic loss due to tick infestation has been estimated as US$14,000–18,000 million annually, and the cost of management of ticks and tick-borne diseases in livestock of India is as high as US$498.7 million per annum (Minjauw and Mc Leod, 2003). *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* are widely prevalent and considered as economically important ixodid ticks infesting dairy animals in India (Ghosh et al., 2007), particularly in Punjab state (Haque et al., 2011; Singh and Rath, 2013).

Presently, the use of chemical acaricides is the most widely employed tick control strategy in India. These chemicals are easily available and are applied on infested animals at frequent intervals leading to their indiscriminate use which has probably contributed to the development of acaricide resistance in ticks (Sharma et al., 2012). Synthetic pyrethroids (SPs), particularly cypermethrin, is the predominantly used commercially available acaricide employed for tick control in Punjab state, India. Beside its application against agriculturally important pests, it is also used extensively for the control of mosquitoes (Ansari and Razdan, 2003; Sharma et al., 2004; Tiwari et al., 2010). Although cattle owners have reported treatment inefficiency in field conditions, only limited data on tick resistance to cypermethrin are currently available from India (Kumar et al., 2006; Sharma et al., 2012; Shyma et al., 2012) and particularly from Punjab state (Singh et al., 2010, 2013). Further, most of the published data available are regarding the resistance status of *R. (B.) microplus*, whereas, reports on the resistance status of *H. anatolicum* are scarce, and no data are available on a comparative basis of the resistance status of single-host versus multi-host ticks. Having in mind the variation in the development of acaricide resistance in different tick types, it becomes essential to generate data for these different tick species for formulation and implementation of effective tick control strategies particularly for countries like India where mixed tick

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infestation is a common feature. The current study was undertaken to generate data on the cypermethrin resistance status of *R. (B.) microplus* and *H. anatolicum* collected from Punjab state, India.

Materials and methods

Study area

Live engorged adult female *R. (B.) microplus* and *H. anatolicum* ticks were collected from cross-bred dairy animals with mixed infestation of both tick species as well as their sheds, from Muktsar and Mansa districts located at the trans-gangetic plain in the state of Punjab (29.30° N to 32.32° N and 73.55° E to 76.50° E) with humid subtropical climatic conditions. Besides, uniform tick infestation patterns, easy accessibility promoted the selection of these regions. Both organized and unorganized farms were selected to collect the samples. A questionnaire was formulated to collect data on frequency, type, and mode of acaricide treatment adopted by the owners and their experiences about the efficacy of commonly used acaricides. The ticks were collected in separate vials, closed with muslin cloth to allow air and moisture exchange, brought to the laboratory, cleaned, labelled and kept at 28 ± 1 °C and 85 ± 5% relative humidity.

Acaricide

Technical grade 100% pure cypermethrin (AccuStandard® Inc., USA) was used to prepare the stock solution in methanol (100 mg/ml). For the experimental bioassay, different concentrations of the acaricide were prepared in distilled water from the stock solution and tested against field samples of *R. (B.) microplus* and *H. anatolicum*.

Adult immersion test

The adult immersion test (AIT) was conducted according to the methods of Drummond et al. (1973) and Sharma et al. (2012). Briefly, preweighed engorged females of *R. (B.) microplus* and *H. anatolicum* were immersed in different concentrations of cypermethrin (100, 200, 300, 400, and 500 ppm) for 2 min and then soaked in filter paper before transferring them into Petri dishes. After 24 h, ticks were transferred to glass tubes covered with muslin cloth and kept in desiccators kept in a BOD incubator maintained at 28 ± 1 °C and 85 ± 5% RH. Ticks which did not oviposit even after 14 days post treatment were considered dead. The control group was treated in a similar manner in distilled water. Each concentration was replicated twice, and 10 adults were used per replication, and the following parameters were compared:

(a) Mortality: recorded up to 14 days post treatment
(b) Egg mass weight laid by the live ticks
(c) Reproductive index (RI) = egg mass wt./engorged female wt
(d) Percentage inhibition of oviposition (%IO) = [(RI control − RI treated)/RI control × 100].

Dose–response data were analyzed by the probit method (Finney, 1962) using Graph Pad Prism 4 software. The lethal concentration of cypermethrin for 50% of ticks (LC_{50} values) was determined by applying regression equation analysis to the probit-transformed data of mortality.

Resistance diagnosis in field samples of ticks

Resistance factors (RF) for field tick samples were worked out by the quotient between LC_{50} of field ticks and LC_{50} of susceptible lines of *R. (B.) microplus* and *H. anatolicum* (Castro-Janer et al., 2009). The LC_{50} values of cypermethrin were 138.5 and 245.9 ppm against acaricide-susceptible reference IVRI-I line of *R. (B.) microplus* (Sharma et al., 2012) and acaricide-susceptible reference IVRI-II line of *H. anatolicum* (Shyma et al., 2012), respectively, both maintained in the Entomology Laboratory, Indian Veterinary Research Institute, for the past 15 years and not been exposed to any acaricides. On the basis of RF, the resistance status in the field populations of *R. (B.) microplus* and *H. anatolicum* was classified as susceptible (RF < 1.4), level I (RF = 1.5–5.0), level II (RF = 5.1–25.0), level III (RF = 25.1–40), and level IV (RF > 40.1) (Sharma et al., 2012).

Results

In the present study, areas with mixed infestations of *R. (B.) microplus* and *H. anatolicum* under similar environmental conditions were covered. Farmers of these areas reported frequent applications of available acaricides particularly cypermethrin without maintaining an optimum concentration for the control of ticks mainly due to low efficacy of most of the marketed products. Hence, uniform treatment was being adopted for the control of both single- and multi-host tick species by the farmers.

Cypermethrin resistance status in Muktsar tick samples

Data on the LC_{50} values of cypermethrin, RF values, and the level of resistance to cypermethrin in the Muktsar samples of *R. (B.) microplus* and *H. anatolicum* are shown in Table 1. The RF value of *R. (B.) microplus* was recorded as 5.48, a level II resistance status, whereas *H. anatolicum* (1.12) had a susceptible status indicating that resistance in the multi-host tick species develops slower. The regression graphs of probit mortality of both tick species plotted against log values of progressively increasing concentrations of cypermethrin are shown in Fig. 1.

The egg mass weights produced by both tick species upon exposure with different concentrations of cypermethrin are shown in Fig. 2. The slope of the egg mass weight of *R. (B.) microplus* (−0.53 ± 0.03) was higher than in *H. anatolicum* (−1.02 ± 0.13). The R^2 values of the egg mass weights of *R. (B.) microplus* and *H. anatolicum* were 0.983 and 0.949, respectively (Table 1). The comparative reproductive index (RI) of both tick species is shown in Fig. 3. The slope of RI of *R. (B.) microplus* was higher than in *H. anatolicum* showing a susceptible resistance status. The slope and R^2 values of *R. (B.) microplus* ticks for RI was recorded as −0.28 ± 0.05 and 0.912, respectively. In case of *H. anatolicum*, the respective values were −0.37 ± 0.01 and 0.996, respectively. The comparative percentage inhibition of oviposition (%IO) in these tick species are shown in Fig. 4. The slope of %IO of *R. (B.) microplus* (57.99 ± 10.38) was lower than in *H. anatolicum* (61.86 ± 2.04), and the respective R^2 values were 0.912 and 0.996, respectively.

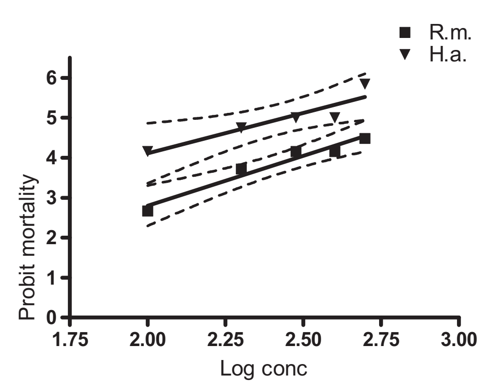

Fig. 1. Dose mortality curves after treatment with cypermethrin in samples of *H. anatolicum* (*H. a.*) and *R. (B.) microplus* (*R. m.*) from Muktsar.