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Studies on evaluation of resistance in common ixodid tick: A preliminary study

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Abstract
Rhipicephalus (*Boophilus*) *microplus*, commonest tick species in India, cause significant economic losses to dairy and leather industries by adversely affecting the milk production and quality of hides. Tick control strategy involves mainly the use of synthetic acaricides which results into development of acaricide resistance. In the present study, the resistance status of *Rhipicephalus* (*Boophilus*) *microplus* against commonly used acaricides was evaluated by standardized method of FAO. The tick samples were collected from cattle owned by livestock holders to determine the status of development of acaricide resistance to Deltamethrin and cypermethrin using larval packet test. The results indicated that isolates of *R* (*B*) *microplus* has developed resistance to deltamethrin and cypermethrin. The data on field status of acaricide resistance will be helpful to adopt suitable strategy to overcome the process of development of resistance in ticks.

Keywords: Acaricide, resistance, *Rhipicephalus* (*Boophilus*) *microplus*

Introduction
Rhipicephalus (*Boophilus*) *microplus*, the tropical or southern cattle tick, is considered to be the most important tick parasite of livestock in the world causing huge economic losses through blood loss, weight loss, stress, irritation, decrease in productivity, depression of immune function, damage to hides and transmission of pathogens (de Castro, 1997) [3]. Application of chemical acaricides is the most common tick control method adopted by the cattle owners because they offer relatively quick and cost-effective suppression of tick populations (Mendes *et al*., 2013) [10]. The synthetic pyrethroids, deltamethrin and cypermethrin, are commercially available in India and at present are two predominant acaricides used for tick control in the country. The continuous and indiscriminate use of acaricides led to the appearance of the resistant tick populations thereby reducing the ability to control them (FAO, 2004; Sharma *et al*., 2012) [4, 14].

The first report of the development of resistance of *Boophilus microplus* to arsenic came from Australia in 1937 (Newton, 1967) [12]. The progressive evolution and development of resistance of ticks to almost all the available acaricides had discouraged the efforts of cattle owners to manage ticks. George *et al.* (2004) [6] documented the selected records of the geographical distribution and the year of documentation of acaricide resistance in populations of tick species.

Resistance has progressively limited the use of chemicals that were used earlier and developed resistance progressively for e.g arsenic, chlorinated hydrocarbons, organophosphate, carbamates and pyrethroids. The fate of remaining acaricides is a matter of great concern and profound discussion as resistance is eventually affecting the usage of chemicals (Nari and Hansen, 1999) [11]. The spectrum of chemical groups to which ticks have evolved resistance continues to widen and necessitates the formulation of guidelines and steps to stop the indiscriminate use of chemical acaricides.

Pyrethrins are natural compounds derived from plants of the chrysanthemum family. Pyrethroids are synthetic adaptations of pyrethrins, specifically designed to be more stable than the pyrethrums and thus have a long lasting effect. Both pyrethrins and pyrethroids are potent neurotoxins. They act on sodium ion channels and thus cause changes in nerve membrane permeabilities to sodium and potassium ions (Weston *et al.,* 2013) [17]. The involvement of esterases (Guerrero *et al.,* 2000) [7], p450s (Chevillon *et al.,* 2007) [2] and Glutathione S-transferases (Konus *et al.,* 2013) [8] in pyrethroid resistance has been demonstrated for many species of ticks.

In a recent study, large scale resistance to organophosphate compound diazinon and synthetic pyrethroids deltamethrin and cypermethrin was experimentally validated in Indian isolates of *Rhipicephalus* (*Boophilus*) *microplus* collected from 6 agro-climatic regions of the country (Kumar *et al.*, 2011 and Sharma *et al.,* 2012) [9, 14].

FAO (2004) [4] has developed the definition of resistance in broad terms as "the ability of a parasite strain to survive and/or to multiply despite the administration and exposure to drug given in recommended dose or above it". Periodic monitoring of the ticks for development of resistance against commonly used acaricides is, therefore, very important for economic livestock production. The present study aims to monitor the status of resistance, if any, in common cattle ticks *Rhipicephalus* (*Boophilus*) *microplus* collected from Lucknow region, Uttar Pradesh.

Materials and methods

Study area and sampling methods

Lucknow, the capital city of Uttar Pradesh, is home to many rural, semi-urban and urban, small and large livestock owners. A questionnaire was formulated to collect the data on frequency, type and mode of acaricide treatment adopted by the respondents/cattle owners, and owners experience about the efficacy of commonly used acaricides.

Methodology

Collection of ticks: A random sampling method was adopted to collect live engorged female *Rhipicephalus* (*Boophilus*) *microplus* ticks from animals and their sheds. The ticks were collected in separate vials, closed with muslin cloth to allow air and moisture exchange and brought to the Parasitology Laboratory. The vials were put in dessicator which was properly humidified by 10% KCl. The dessicator was kept in incubator at $28^0 \pm 1\ ^0$C

Maintenance of Tick stages in laboratory

a) Egg laying: After one week, the ticks started laying eggs in several stocks up to 20 to 25 days.

b) Hatching of Eggs: After completion of the egg laying process, eggs started hatching into larvae in 5-7 days.

Test Acaricide tested: Butox (Deltamethrin E.C 1.25%) and Cypermass (Cypermethrin E.C 25%)

Preparation of Acaricide for Assay: Butox (Deltamethrin E.C 1.25%) and Cypermass (Cypermethrin E.C 25%) were used to prepare the stock solutions of 10,000 ppm in distilled water. For the experimental bioassay, different concentrations of the acaricide (25, 50, 75, 100 and 125 ppm) were prepared from the stock solution in distilled water and tested against field isolates of *Rhipicephalus* (*Boophilus*) *microplus.*

Bioassay

Larval Packet Test (LPT)

The Larval Packet Test described by Food and Agricultural Organization (FAO, 2004) [4] was used to determine the efficacy of chemical. The LPT was first described by Stone and Haydock (1962) [16]. Whatman filter paper 1 was used to prepare packets; paper was cut and packets were prepared. The prepared packets were dipped for two minutes in 2ml of each concentration and kept for drying. When the packet was completely dried, 50 larvae were put in each packet with the help of brush. 5 replicates of each concentration were prepared. The top of each packet was sealed with adhesive tape and the packets were placed in a desiccator kept in BOD incubator maintained at $28^0 \pm 1\ ^0$C and 85±5% RH. After 24 hours, the mortality of larvae was observed and recorded.

Mortality = Total no. of tick larvae in packet –live tick larvae

Percentage mortality was calculated for each replicate of concentration with the following formula and then mean Percentage % mortality was obtained.

$$\text{(\%) Percentage Mortality} = \frac{\text{No. of dead larvae}}{\text{Total No. of larvae}} \text{ X } 100$$

Data analysis

Calculation of LC_{50} and LC_{95} (Probit Analysis): In order to evaluate the results of bioassays in living organisms, probit analysis method is adopted. The tick larvae had been exposed to the different concentration deltamethrin and cypermethrin. Therefore, a binary response has been observed *i.e*; the death and survival of larvae after treatment with deltamethrin and cypermethrin. Probit analysis (Finney, 1962) [5] was applied to calculate LC_{50} and LC_{95} values.

Acaricide susceptible ticks for reference

The reference data for the present study was taken from the literature available (Shyma *et al.,* 2013) [15] and used as the standard to assess susceptibility/resistance status in ticks collected from study area.

Results and discussion

The purpose of the present study was to determine whether there is any development of acaricide resistance in the tick *Rhipicephalus* (*Boophilus*) *microplus* and to estimate the quantum of acaricidal resistance, if any, persisting in different field isolates. The results of the survey work conducted in the study area shows that the tick infestations is a major problem of cattle and other livestock as well. It has been observed during the study that owners/holders and farmers mainly use chemical acaricide to treat their animals from tick infestation. They commonly use deltamethrin and cypermethrin to control ticks from their animals.

The LPT was adopted to study the evaluation of anti-tick activity of the chemicals. It takes 5–6 weeks to complete. Commercial formulation Butox (Deltamethrin E.C 1.25%) and Cypermass (Cypermethrin E.C 25%) were used for the present study. The stock solutions (10,000 ppm) of both acaricide and the working concentrations were prepared with distilled water. The results are depicted in Table 1.

The present study has aimed to monitor the status of resistance, if any, developed for deltamethrin and cypermethrin in cattle ticks *Rhipicephalus* (*Boophilus*) *microplus* collected from Lucknow region, Uttar pradesh. Larval Packet Test (LPT) recommended by FAO (2004) [4] was followed for the evaluation and the results were analyzed by probit analysis (Finney, 1962) [5] and LC_{50} and LC_{95} values of deltamethrin was calculated. The LC_{50} and LC_{95} values of deltamethrin for the ticks isolated from Lucknow were 58.8 ppm and 234.42 ppm, respectively and for cypermethrin, it is 165.95 ppm and 7244.35 ppm respectively (Table 1). Regression graph for both the chemicals was also plotted and regression equation and R^2 value was also obtained. The same are represented in Figure 1 and 2.

In case of IVRI I reference susceptible tick line, the LPT results have shown that the LC_{50} and LC_{95} values of deltamethrin were 11.8 ppm and 35.5 ppm, respectively. While in case of cypermethrin, the LC_{50} and LC_{95} values were 242.4 and 350.7 ppm, respectively (Shyma *et al.*, 2013) [15]. It is seen from the results cited from literature that there is large difference in the LC_{50} and LC_{95} value of chemicals exposed to susceptible and tested ticks of study area. This may be an indication of the development of resistance in the ticks collected from Lucknow region. However, for more conclusive results further studies are needed.

A wide variation in the LC_{50} values of these acaricides against reference lines of *Rhipicephalus* (*Boophilus*) *microplus* maintained in different laboratories was cited in the literature. There is wide variation in results and this has been attributed to the use of different reference tick lines and different types of bioassay (AIT or LPT) (FAO, 2004) [4]. The LC_{50} values of deltamethrin against different strains were determined as 120 ppm in susceptible Milargo strain (Argentina) (Aguirre *et al*., 2000) [1] and 40 ppm in Yeerongpilly strain (Australia) (Nolan *et al*., 1989) [13]. While the LC_{50} value of cypermethrin was estimated as 210 ppm in Milargo strain (Argentina), 370 ppm in Yeerongpilly strain (Australia) (Nolan *et al.*, 1989) [13] and 400 ppm in Porto Alegre strain (Brazil). The wide variation in the LC_{50} values in cited literature strongly suggest that there is an urgent need for the generation of base line data of representative tick species of the country before working out the resistance status in ticks of the respective country. The results of the present study will be a useful tool for monitoring of resistance status of synthetic pyrethroid in tick.

The investigation by Shyma *et al.* (2013) [15] showed that the level of acaricide resistance is comparatively higher in the one-host tick, *Rhipicephalus* (*Boophilus*) *microplus* than in the three host tick, *Hyalomma analoticum analoticum* which corroborates the observations made on global basis (Wharton and Roulston, 1970) [18]. Existence of high acaricidal resistance in *Rhipicephalus* (*Boophilus*) *microplus* populations has also been documented in Rajasthan (Sharma *et al.,* 2012) [14]. They observed that a much larger fraction of the total population of one host ticks remained under chemical challenge because of their shorter life cycle and host specificity to domestic cattle. However, a three-host tick, *Hyalomma* species, may come in contact with environmental residues of the insecticides and thus contribute to increasing the selection pressure (Shyma *et al*., 2013) [15].

The overall prevalence of elevated level of resistance may be a result of widespread use of tick control with pyrethroids particularly in cross bred cattle (85%) in the farms. Due to more susceptibility to tick infestations, cross-bred cattle were always subjected to more frequent treatment with acaricides.

Table 1: Mean % mortality and LC_{50}/LC_{95} value on exposure to deltamethrin and cypermethrin after 24 hr.

Concentration	Mean % mortality on exposure to Deltamethrin after 24 hr	Mean % mortality on exposure to Cypermethrin after 24 hr
25ppm	26	22.2
50ppm	24	27
75ppm	65	33.2
100ppm	68	43
125ppm	79	46.8
LC_{50}	58.8 ppm	165.95 ppm
LC_{95}	234.42 ppm	7244.35 ppm
P>0.05 N.S		

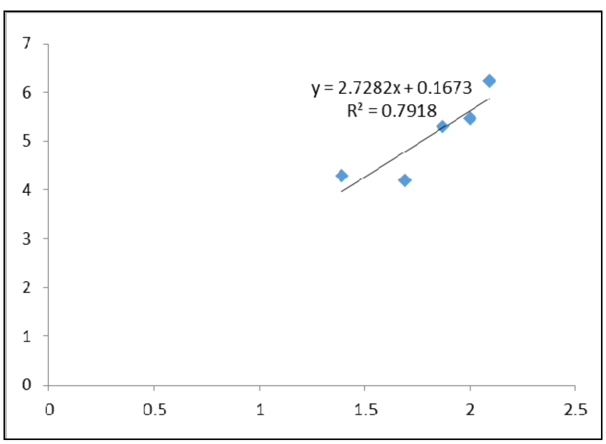

Fig 1: Probit mortality x log concentration plots from *Rhipicephalus* (*Boophilus*) *microplus* Larvae submitted to Larval Packet test with Deltamethrin.

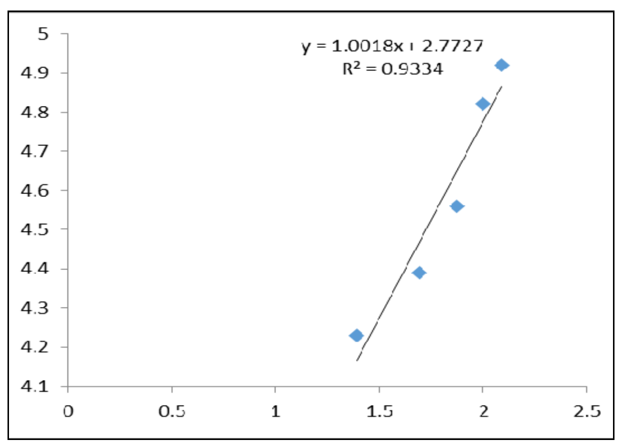

Fig 2: Probit mortality x log concentration plots from *Rhipicephalus* (*Boophilus*) *microplus* Larvae submitted to Larval Packet test with Cypermethrin.

Conclusion

The purpose of the present study was to monitor the status of acaricide resistance of ticks collected from the study area against deltamenthrin and cypermethrin in the study area. As per the literature available, the value of LC_{50} and LC_{95} is far less in case of susceptible tick line available than in the results obtained from ticks isolated from Lucknow. There is also an urgent need for continuous monitoring of acaricide resistance in field situation for strategic application of available acaricides and for maintaining the life span of the product. Use of vaccines, synthetic and botanical acaricides in combination and educating the farmers by launching extension programs about recommended tick control practices as strategic and/or tactic measures for the control of cattle ticks would be rewarding. Integration of currently available options for the management of drug resistance is an important operational and research priority.

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