Acaricide Resistance Status of *Rhipicephalus (Boophilus) microplus* against Cypermethrin and Deltamethrin in Uttar Pradesh.

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Abstract: *Rhipicephalus (Boophilus) microplus* is a common tick species found in the animals belonging to southern and tropical areas. It causes huge economic loss in the livestock. Infestation due to tick species usually leads to weight loss, blood loss, hair loss, irritation, inferior quality of hides, reduction in milk and meat production, weakness in immune system and transmission of pathogens. People belonging to rural areas commonly use synthetic acaricides for the tick removal and the animals slowly develop resistance to them. The present study, hence was taken up to asses the status of acaricide resistance in ticks. The tick samples were collected from animal sheds, dairies, farms and small animal holders. Larval Packet Test was conducted as per the method standardised by FAO, to assess the status of acaricide resistance in the tick. Two commercial acaricides, cypermethrin and deltamethrin were used against tick infestation in domestic animals. Results of the present research revealed that *Rhipicephalus (Boophilus) microplus* tick species have developed resistance to both acaricides. The present findings will help in the assessment of resistance status of acaricides in ticks and to deviseand adopt better management practices to reduce the losses caused due to tick infection in livestock.

Keywords: **Transmission, Pathogen, Synthetic acaricides, *Rhipicephalus (Boophilus) microplus*.**

I.INTRODUCTION

In India, pyrethroides, cypermethirin and deltamethrin, are two predominating acaricides used for predominating acaricides which are used for tick controlling system. The regular use of acaricides leads to increased resistance (Sharma *et al.*, 2012 and FAO, 2004).Resistance has restricted the use of chemicals considerably that were earlier used and developed resistance afterwards; e.g. arsenic, carbamates, chlorinated hydrocarbons, organophosphates and pyrethroides etc. The fate of remaining acaricides is a matter of great concern and discussion as resistance will increase in the long run and will limit the usage of acaricides (Nari and Hansen, 1999).

The development of resistance of *Rhipicephalus (Boophilus) microplus* to arsenic was first reported in Australia in 1937 by Newton, (1967). The increasing tick resistance in animals against all the acaricides which are available in the stores had discouraged the livestock holders in their rearing(Kunj and Kemp, 1994; Wharten and Roulston, 1970).

Some ethno-veterinary plants are also used for controlling the ticks in animals. Isolated plant extracts from *viz.* Pyrethroids which are natural compounds derived from plant of chrysanthemum family are found to be strong neurotoxins. It acts on sodium and potassium ions (Weston *et al.*,2013). In recent research, Sharma *et al.*, 2012 and Kumar *et al.*, 2011, had been reported great resistance to diazinon (organophosphate drug) and synthetic pyrethroids such as deltamethrin and cypermethrin which were experimentally confirmed in field isolates of *Rhipicephalus (Boophilus) microplus* (Sharma *et al.*, 2012 and Kumar *et al.*, 2011).

1.1 COMMON TERMS

Resistance

According to FAO (2004) “the ability of a parasite strain to survive or/ and to multiply despite the administration and exposure to drug given in recommended dose or above it”. It is generally considered as a failure of a drug to inhibit parasitism. In other words definition of resistance is a modified receptiveness in the stimulus for a drug (Corley *et al.*, 2013 and Sangster, 2001).

a) Acquired resistance

Acquired resistance is the result of continuous reduction in sensitivity to drug in the long duration of time and it is also heritable (Chapman 1997 and Meyer *et al.*, 2012). There is a direct relationship between degree of resistance and concentration of the drug. Tick resistance increases by giving continuously high exposure of a drug.

b) Cross- Resistance

Cross resistance in ticks is defined as resistance to various acaricides with a similar mode of action. Pattern of cross resistance has been observed between two organophosphates (diazinon and coumaphos) and one carbamate (carbaryl) in many strains of *Rhipicephalus (Boophilus) microplus* (Li *et al.*,2005).

c) Multiple Resistance

Resistance for many drugs although have different mode of action is known as multiple resistance Foil *et al.*,(2004) concluded in their research a significant occurrence of multiple resistance in southern cattle ticks due to different classes of acaricide including organophosphates, formamidines (amitraz), chlorinated hydrocarbons (DDT).

The present study was designed to generate data to understand and record the status of resistance of acarisides in common animal tick species, i.e., *Rhipicephalus (Boophilus) microplus* which was collected from five zones (East, West, North, South and Central zone) of Uttar Pradesh, state of India.

II. Materials and Methods

2.1 Study Area and Sample Collection

During study period, a survey was conducted for collection of engorge female ticks (*Rhipicephalus (Boophilus) microplus*) across many rural, semi- urban and urban areas from sheds of farms and dairies, comprising cross bred cattle as well as buffaloes from different zones of Uttar Pradesh (table 1). In Uttar Pradesh, it is quiet evident through many researchers that many small and large livestock holders generally use semi- intensive and intensive rearing system to rear the animals and mostly farmers depends on agriculture and animal husbandry for their livelihood. During survey a questionnaire was prepared to collect the information, method and type of acaricides used to control tick species by the livestock owners and to know about potency of acaricides.

2.2 Collection of engorged female ticks of *Rhipicephalus (Boophilus) microplus*

Collection of ticks was done using random sampling method from different zones of Uttar Pradesh state of India. Collected engorged female tick species, *Rhipicephalus (Boophilus) microplus* were kept in separate vials, for exchange of moisture and air, mouth of vials were closed with muslin cloth, brought to the Parasitology Laboratory, Department of Applied Animal Sciences, B.B.A. University Lucknow. These vials were put in humidified dessicator with 10% KCL and dessicator was kept at $28^0 \pm 1^0$ in incubator.

III. Maintenance of ticks in laboratory up to larval stage for Larval Packet Test (LPT)

3.1 Process of Larval Hatching

a) Egg Laying: After 7-8 days the engorged ticks started laying eggs.

b) Egg Hatching: After egg laying, eggs hatched to in larvae in 6-7 days.

3.2 Acaricides used for the test: The following acaricides were used for conducting the test.

a) Cypermass (Cypermethirin E. C. 25%)

b) Butox (Deltamethrin E. C. 1.25%)

3.2 Preparation of Acaricides for Bioassay

For the experimental bioassay stock solutions of 10,000 ppm in distilled water was prepared with the Cypermass (Cypermethrin E.C. 25%) and Butox (Deltamethrin E. C. 1.25%). Desired grades of different concentrations of the acaricides (25, 50, 75, 100 and 125 ppm) were prepared in distilled water from the stock solutions and tested against the tick species *Rhipicephalus (Boophilus) microplus*.

3.3BIOASSAY

3.3.1 Larval Packet Test (LPT)

Larval packet test was conducted as per the procedure standardized by Food and Agricultural Organization (FAO 2004). LPT is used to determine the potency of acaricides. Which was firstly reported by Stone and Hydock (1962). Some other researchers also described this method (Castro- Janer *et al.*, 2009). Packets were prepared using filter paper and then packets were impregnated in 2 ml of each concentration of chemical and then are dried. Fifty larvae were put in each dried packet with five replicates of each concentration (25, 50, 75, 100 and 125ppm). Mouth of packets was sealed with a tape. Packets were kept in dessicator and the dessicator was kept in BOD incubator maintained at 28^0c ± 1^0cand relative humidity 85 ± 5%. Larval mortality was recorded every day and calculated using the formula-

Tick larvae Mortality = Total no. of tick larvae in packet – Live tick larvae

The percentage mortality was also recorded using formula-

$$\text{Percentage mortality (\%)} = \frac{\text{No.of dead larvae}}{\text{Total no.of larvae}} \times 100$$

3.3.2 DATA ANALYSIS

3.3.3 Probit Analysis (Calculation of LC 50 and LC 95)

For the evaluation of bioassay in living organisms probit analysis method was used. Determination of dose response data on tick larvae was analysed using two chemicals cypermethrin and deltamethrin. Binary response i.e. death and survival of tick larvae has been recorded after treatment of cypermethrin and deltamethrin. Calculation of LC 50 and LC 95 values was determined by probit analysis method (Finney,1962; Hadded, 2010).

Among the groups, the variations in mean values of data were analyzed usingStudent's t- test method (Snedecor and Cochran, 1968).

3.3.4 REFERENCE FOR ACARICIDE SUSCEPTIBLE TICKS

Data of the reference for this study was taken from the literature (Shyama *et al.*, 2013) and accordingly, observation of resistance status in ticks was used as the standard.

IV.RESULTS AND DISCUSSION

Present study was designed to determine the status of acaricide resistance in the tick *Rhipiciphalus (Boophilus)microplus*. During survey period, high tick infestation was recorded in rainy season. Results of survey study revealed high tick infestation in the livestock. Animal holders also reported that less attention was paid to the problem of tick infestation in animals. In this study it also has been reported that in rural areas, farmers/ animal holders generally use synthetic acaricides, for tick removal from the body of animals. cypermethrin and deltamethrin were found to be very commonly used acaricides by farmers/ animal holders, since these are very easily available in the market.

Larval Packet Test was conducted to evaluate the effectiveness of anti-tick property of the acricides/ chemicals. The total duration of LPT test was 5-6 weeks. Two commercial acaricides- Cypermass (cypermethrin E.C. 25%) and Butox (deltamethrin E.C. 1.25%) were used in the present study. The stock solutions (10,000 ppm) of cypermethrin and deltamethrin as well as working concentrations were prepared with the help of distilled water and the results are presented in the table 1.

Table 1. Mean percentage mortality (%) and LC50/ LC 95 value after 24 hour exposure with cypermethrin anddeltamethrin.

Concentration (ppm)	**Mean percentage (%) mortality on exposure to cypermethrin after 24 hr.**	**Mean percentage(%) mortality on exposure to deltamethrin after 24 hr.**
25	47%	54%
50	60%	63%
75	69%	71%
100	77%	81%
125	84%	87%
LC 50	29.847ppm	25.106 ppm
LC95	422.458 ppm	328.031 ppm
P Value	P= .0010 (≤ 0.05 Significant)	P= .0013 (≤ 0.05 Significant)

Larval Packet test was performed as per the procedure standardised by Food and Agriculture Organization (2004). Tick mortality data were evaluated through probit analysis (Finney, 1962) and LC50, LC95 values of cypermethrin and deltamethrin were determined. The LC50 and LC95 values of cypermethrin for the ticks collected from different zones of Uttar Pradesh were 29.847ppm and 422.458 ppm, respectively. The LC50 and LC95 values of deltamethrin were recorded to be25.106 ppm and 328.031 ppm, respectively (Table 1). Regression graph was also plotted for both the chemicals, regression equation and R^2 values were also obtained. (Fig. 1 and Fig. 2).

In the Larval Packet Test a 100 % tick mortality reference IVRI I line was observed. Results revealed that LC50 and LC95 values of cypermethrin were 242.2 ppm and 350.7 ppm whereas in case of deltamethrin the LC50 and LC95 values were 11.8 ppm and 35.5 ppm, respectively (Shyama *et al.*,2013). Results indicated a large difference in the LC50 and LC95 values of chemicals. Present study also showed development of resistance in the ticks collected from Uttar Pradesh region.

Similar findings were also reported in the study conducted by Kaur, *et al.,* (2017), who found a huge difference in the development of acaricide resistance in the ticks and observed values of LC50 and LC95 with respect to deltamethrin that was 58.8 ppm and 234.42ppm., respectively and in case of cypermethrin it was165.95ppm and 7244.35 ppm, respectively.

Similar results were reported in the development of acaricide resistance status (Shyma *et al.*, 2012; Sharma *et al.*, 2012 and Kumar *et al.*, 2011). Available literature cites a big difference in the LC50 values of these acaricides against reference lines of *Ripiciphalus (Boophilus) miroplus.*

The study of Shyama *et al.*, 2013showed comparatively higher acaricidal resistance in one host tick *viz. Rhipiciphalus (Boophilus) microplus*, in comparison of, *Hyalomma anatolicum anatolicum*.. They found higher fraction of one host ticks which remained alive even under chemical challenges since this species have shorter life cycle and specificity of host to domestic animals.

Variations in the LC50 and LC95 values in available literature, suggests that there is an urgent need for the generation of standard data of respective tick species in the country. The results of this study will be very much useful for the assessment of resistance status of acaricides in ticks.

The increased level of acaricide resistance in the tick species found in overall prevalence is due to popular tick control methods used in organised and unorganised animal farms in the study area. Rearers generally used pyrethroids in cross breed animals and it was observed by many that near about 85% cross breed cattles were treated with these chemicals very frequently for tick control.

V. CONCLUSION

The objective of the present study wasto determine the status of acaricide resistance of field isolates collected from the study area against two common acaricides used i.e. cypermethrin and deltamethrin. The values of LC50 and LC95 were found to be less in respect to susceptible tick line available than in the results observed from tick isolates from Uttar Pradesh state of India.

Goncalves *et al.*,(2007) and Stratton and Corke (1982) suggested that the continuous use of organic solvents increases adsorption of acaricide over the surface area of target biological sample and also augments penetration of active ingredients of the acaricide across the exoskeleton. Sharma *et al*.,(2012), studied the safety levels of acetone and methanol up to the level of 50% as well as effect of various solvents on the biology of reference IVRI-I line of *Rhipiciphalus (Boophilus) microplus.*

The increment of immunity in domestic animals is also an important factor for tick control. Use of vaccines and ethno-veterinary plants in place of acaricides is another method to reduce the use of acaricides.Nutritional aspect is an important factor in resistant management according to O' Kelly and Seifert, 1969. Defective T-cell which is important for immune response is also affected by protein energy deficiency.Continuous monitoring of animal ticks for the cause of resistance against various classes of chemical acaricides has a great role in the supervision of resistance and development of an effective drug. Use of synthetic and plant based acaricides as well as vaccines in combination and also educating the farmers by providing them appropriate knowledge about tick control practices for controlling animal ticks will be highly beneficial.

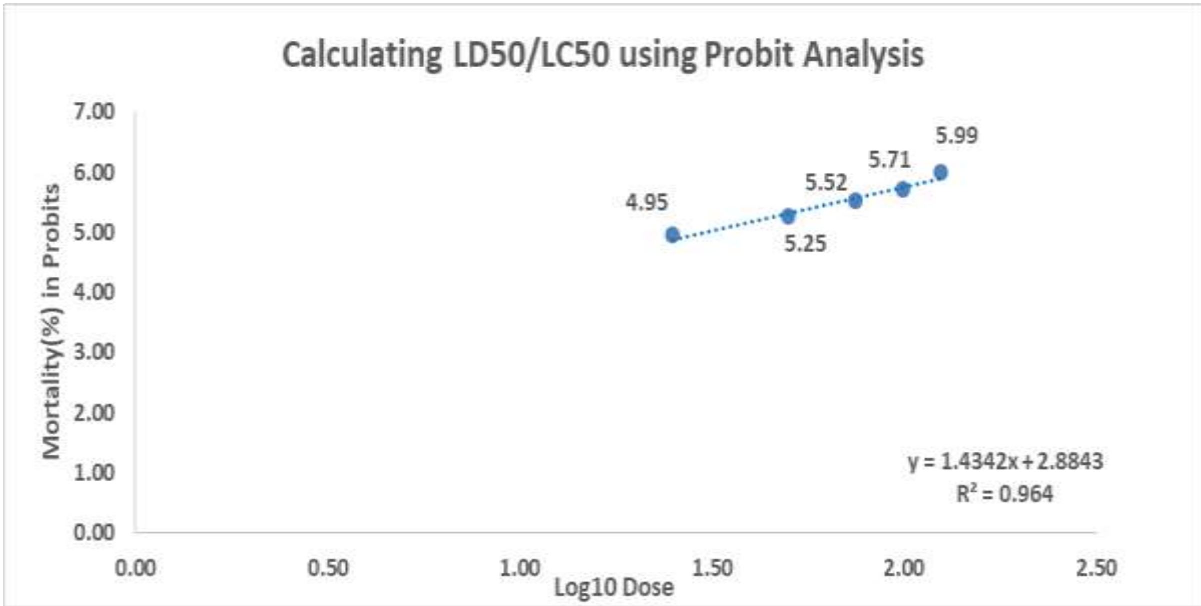

Figure 1. Probit mortality x log concentration plots from *Rhipicephalus* (*Boophilus*) *microplus* Larvae introduce to Larval Packet test with cypermethrin.

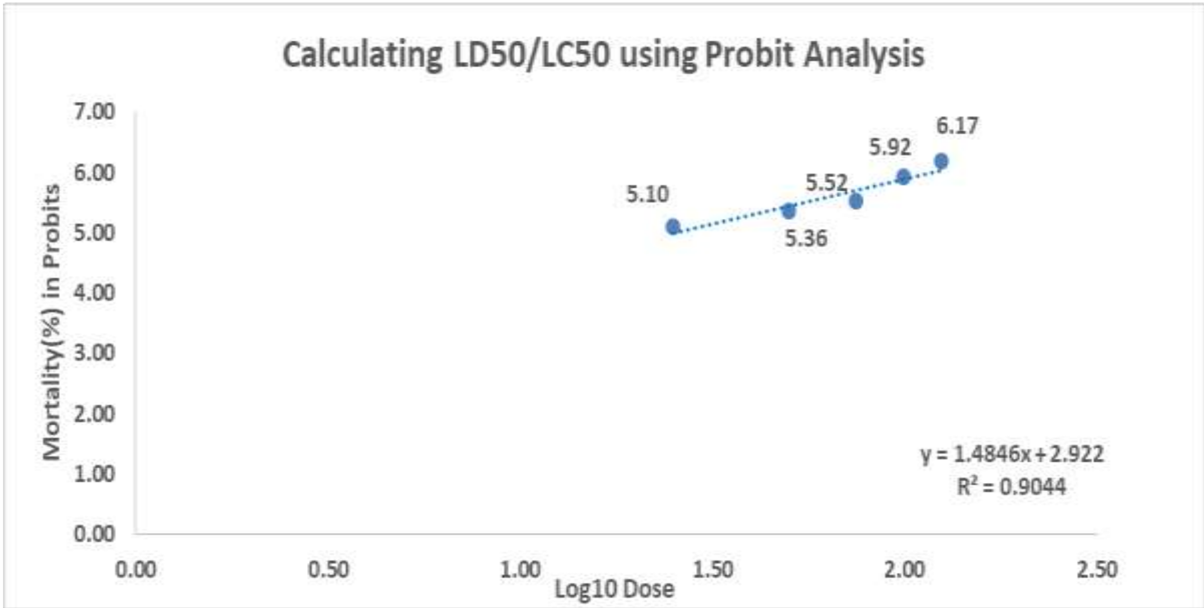

Figure 2. Probit mortality x log concentration plots from *Rhipicephalus* (*Boophilus*) *microplus* Larvae introduce to Larval Packet test with deltamethrin.

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