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COMPARATIVE EFFICACY OF TWO SYNTHETIC PYRETHROIDS AGAINST *RHIPICEPHALUS (BOOPHILUS) MICROPLUS*

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ABSTRACT: A study was conducted to estimate the efficacy of market available formulations of two synthetic pyrethroids namely cypermethrin and fenvalerate. Fully engorged females of *Rhipicephalus (Boophilus) microplus* were collected from different locations of North India. Larval Packet Test (LPT) was conducted on 14 to 21 days old larvae of these tick samples. Ticks reared in the Department of Veterinary Parasitology, GB Pant University of Agriculture & Technology were used as reference susceptible population. Different dilutions of cypermethrin and fenvalerate were prepared in 2:1 solution of trichloroethylene and olive oil as per FAO recommendation. The same solution was used as control. LPT bioassay revealed tick population of Dehradun to be least susceptible, with highest LC_{50} values against both cypermethrin (0.03459% Active Ingredient (AI)) and fenvalerate (0.89802% AI). LC_{50} value of cypermethrin was lowest for Bheemtal (0.0006% AI) and for fenvalerate it was for Pantnagar (0.01817% AI). Four of the tick populations were resistant to cypermethrin (Resistance Factor (RF) > 5) and only tick populations of Dehradun was resistant to fenvalerate. The above study indicates that there is a rise in resistance against the two commonly used synthetic pyrethroids in tick populations of these regions and there is a variation in efficacies of the two acaricides. Thus to prevent the emergence of resistant tick populations in this region, acaricides must be used judiciously and there should be frequent monitoring of efficacy of these acaricides.

KEY WORDS: synthetic pyrethroids; *Rhipicephalus (Boophilus) microplus;* cypermethrin; fenvalerate; active ingredient (AI); LC_{50}; resistance factor

INTRODUCTION

Rhipicephalus (Boophilus) microplus is an endemic pest in tropical and subtropical regions of the world causing huge economic losses to the cattle industry and acts as a disease vector, transmitting the causative agents of babesiosis and anaplasmosis (De Castro 1997). Cattle producers incur high costs of acaricides and drugs to improve the general health of their cattle and to prevent outbreaks of tick-borne cattle diseases. Large populations of *R. (B.) microplus* are currently widespread in India and this is negatively affecting the competitiveness of Indian cattle producers.

In India alone the cost of tick born diseases (TBDs) has been calculated as Rs 2000 crore annually (Minjauw and Mc. Lead 2003). Traditional control methods of ticks in India involve intensive use of acaricides particularly synthetic pyrethroids, a practice which has resulted in the selection of resistant populations whose control presents a major problem to cattle owners.

There had been an increase in reports of reduced efficacy of market available synthetic pyrethroids against *R. (B.) microplus* from the Northern regions of India. Keeping in mind the present scenario a study was conducted to assess the susceptibility of *R. (B.) microplus* against two synthetic pyrethroids namely cypermethrin and fenvalerate.

MATERIALS AND METHODS

R. (B.) microplus ticks reared at the Department of Veterinary Parasitology, GB Pant University of Agriculture & Technology were used as reference susceptible population. The ticks are reared by the department free from any contact with acaricides and are regularly screened for susceptibility to synthetic pyrethroids.

Engorged female tick samples of *R. (B.) microplus* were collected from 6 different locations of Northern state of India, out of these 2 regions namely Pantnagar (243.8 m above sea level), Rudrapur (208 m above sea level) are located in the plains of the state, whereas Ramnagar (1729 m above sea level), Bheemtal (1375 m above sea level), Almora (1638 m above sea level) and Dehradun (700 m above sea level) are in hilly regions of the state.

The ticks collected from different locations were held separately in groups of five each in glass vials and kept in BOD incubator maintained at 27±2º C and 85–92% RH for egg laying. Larval Packet Test (LPT) was performed to ascertain the susceptibility of the ticks collected from above mentioned regions.

Fourteen to twenty one day old larvae were tested for susceptibility by using triplicates of about 100 larvae each exposed to filter papers impregnated with different concentrations of market available cypermethrin (Tick-out®, 10%) and fenvalerate (Ticomax®, 20%). Two parts of trichloro-ethylene and one part of olive oil was used as diluents. For this test, following concentrations of cypermethrin w/v: 0% (controls, filter papers impregnated with diluents only); 0.00125%, 0.0025%, 0.005%, 0.010%, 0.020% and 0.040% and for fenvalerate w/v: 0%; 0.015%; 0.03%; 0.06%; 0.12%; 0.24% and 0.48% were used. After 24 hrs of incubation the packets were observed for live and dead counts to obtain the larval mortality data. Each testing dose was tested in triplicate and the average of dead and live larvae was scored. The larval mortality in Larval Packet Test at a given testing dose was calculated with the formula.

$$\% \text{ Mortality} = \frac{\text{Dead larvae}}{\text{Total larvae}} \times 100$$

The data was discarded when mortality in the control group was more than 10%. Abbots formula (Abbot 1925) was applied when mortality in the control group ranged between 5 to 10%.

The larval mortality data was subjected to probit analysis for calculating LC_{50} (lethal concentration to 50% of tick larvae tested), LC_{99} (lethal concentration to 99% of tick larvae tested) values along with 95% confidence lines and slope of probit lines. The data was analysed by computer program software based on Finney (1971). Resistance factor (RF) was obtained by comparing the LC_{50} of field strains with the LC_{50} of reference susceptible strain.

RESULTS

LPT bioassay results revealed highest LC_{50} values of cypermethrin (0.03459% active ingredient) and fenvalerate (0.89802% AI) for Dehradun population of ticks. This was followed by ticks collected from Rudrapur for cypermethrin (0.0052% AI) and for fenvalerate it was for tick samples of Almora (0.07015% AI). LC_{50} of cypermethrin was lowest for ticks collected from Bheemtal (0.0006% AI) and of fenvelerate it was for ticks of Pantnagar (0.0181% AI).

The LC_{50} confidence interval (CI 95%) of cypermethrin was widest for Pantnagar sample of ticks, upper limit being 152.38% more than the lower limit. It was narrowest for Dehradun ticks (16.56%). For fenvalerate CI 95% was widest for ticks of Bheemtal (228.71%) and narrowest for the Dehradun tick population (18.15%).

Against cypermethrin, Dehradun tick population showed highest resistance, with a Resistance Factor (RF) value of 44.99, whereas ticks of Pantnagar, Rudrapur and Almora were 6.03, 6.8 and 5.79 times more resistant than reference susceptible strain respectively. It was least for tick populations of Bheemtal, RF being 0.883.

The highest RF value for fenvalerate, 61.76 were for tick samples from Dehradun, followed by ticks of Almora with a value of 4.82. Lowest RF value of 0.0983 was for ticks of Bheemtal.

Thus LPT bioassay results revealed five tick population samples namely Dehradun (RF 44.92), Rudrapur (RF 6.80), Pantnagar (6.02) and Almora (RF 5.79) to be resistant against cypermethrin (RF> 5). Tick population of Ramnagar (RF 1.36) and Bheemtal (RF 0.88) were susceptible (RF< 3). In case of fenvalerate Dehradun tick samples (RF 61.76) were resistant, tick samples of Almora (RF 4.82) was tolerant (RF 3 to 5), whereas tick populations of Pantnagar (1.249), Rudrapur (1.269), and Ramnagar (1.633) and Bheemtal (0.0983) were susceptible.

DISCUSSION

LPT bioassay results revealed wide variations in susceptibility of different tick populations to both the synthetic pyrethroids tested. Dehradun ticks exhibited least susceptibility and were resistant to both the acaricides. Vatsya et al. (2009) reported the same in the tick populations of various regions of Northern state of India against cypermethrin. Rosario-Cruz (2005) reported variations in susceptibility of Mexican tick strains to deltamethrin, cypermethrin and flumethrin. This variation may be because Tick-out® (cypermethrin) is used much more frequently and even at concentrations higher than the recommended, fenvalerate is being used on a lesser scale. Ticks collected from Bheemtal were highly susceptibile to cypermethrin and fenvalerate, the LC_{50} value being lower than the reference susceptible population. This may be due to the fact that in this region tick burdens on animals are low and are controlled by hand picking, burning and use of indigenous herbal preparations. Aguirre et al. (2000) obtained LC_{50} of deltamethrin and cypermethrin for Milagro strain of Argentina as 0.012% and 0.0535% respectively, which were lower than the LC_{50} values for Porto Alegre reference susceptible strain.

This heterogeneity of toxic response of ticks to different synthetic pyrethroids suggests that there are likely multiple mechanisms of resistance present in *R.(B.) microplus*, so that each have their own synthetic pyrethroid selective metabolising

Table 1.
Efficacy of cypermethrin against *R. microplus* collected from different locations of North India

Location	Number of larvae	Slope	Chi-square	Standard error (SE)	LC_{50}	C I (95%)	LC_{99}	C I (95%)	Resistance Factor (RF)
Reference Population	2100	1.5855	63.0783	0.0881	0.0007	0.0007–0.0009	0.0228	0.0167–0.0311	1
Pantnagar	2100	1.8949	130.7324	0.1424	0.0046	0.0021–0.0053	0.2823	0.1124–0.3216	6.0285
Rudrapur	2100	1.5179	75.8621	0.0570	0.0052	0.0047–0.0057	0.1797	0.1330–0.2426	6.8051
Ramnagar	2100	1.6015	84.9672	0.0652	0.0010	0.0009–0.0011	0.0301	0.0239–0.0380	1.3636
Bheemtal	2100	1.7559	42.8567	0.1245	0.0006	0.0005–0.0008	0.0145	0.0099–0.0209	0.8831
Almora	2100	1.3052	40.2199	0.0547	0.0044	0.0040–0.0049	0.2721	0.1875–0.3945	5.7922
Dehradun	2100	1.8252	195.2841	0.0573	0.0345	0.0320–0.0373	0.6540	0.5313–0.8051	44.9220

Table 2.
Efficacy of fenvalerate against *R. microplus* collected from different locations of North India

Location	Number of larvae	Slope	Chi-square	Standard error (SE)	LC_{50}	C I (95%)	LC_{99}	C I (95%)	Resistance Factor (RF)
Reference Population	2100	1.4854	146.3357	0.0802	0.0145	0.0121–0.0174	0.5385	0.3926–0.7387	1
Pantnagar	2100	1.6827	126.8543	0.0730	0.0181	0.0158–0.0286	0.4405	0.3514–0.5521	1.2496
Rudrapur	2100	1.2687	82.8684	0.0566	0.0184	0.0156–0.0216	1.2628	0.9339–1.7073	1.2696
Ramnagar	2100	0.9094	28.5622	0.0481	0.0237	0.0196–0.0287	8.6657	5.1029–14.716	1.6334
Bheemtal	2100	1.0916	68.7843	0.08174	0.0014	0.0007–0.0023	0.1929	0.09926–0.3751	0.0983
Almora	2100	1.0392	54.9700	0.0484	0.0701	0.0615–0.0799	12.244	7.5714–19.8022	4.8246
Dehradun	2100	1.6673	370.4482	0.0540	0.8980	0.8261–0.9761	22.423	17.6866–28.4287	61.7620

enzymes or different types of target site insensitivity properties (Rosario-Cruz et al. 2009). Swaid et al. (2012) reported the role of esterase enzymes in varied susceptibility *R. (B.) microplus* of to deltamethrin.

LC_{50} and LC_{99} estimates and confidence limits provide a clue about the resistance status of the tick population of a particular area (Vieira-Bressan et al. 1999). These values however provide no information about the number of resistant and susceptible individuals in a tick population and also do not give any clue about the factors involved in resistance development. Though the values offer no information regarding the number of resistant individuals in a population, these values indicate the existence of resistance.

Among various tick species *R. (B.) microplus* has shown the greatest tendency towards development of resistance, this probably could be because it is a single host tick and spends all its stages (larva, nymph and adult) stages on the single animal thus remaining exposed to acaricides for prolonged periods (Baffi et al. 2007).

These preliminary studies of sensitivity of *R. (B.) microplus* to synthetic pyrethroids are indicative of emerging acaricide resistance against cypermethrin, but the level of resistance is lower for fenvalerate. The above study indicates that synthetic pyrethroids should be used judiciously and there should be frequent monitoring of efficacy of these acaricides in order to avoid resistance development in tick populations against these acaricides and to prolong their effective life.

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