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In vitro detection of synthetic pyrethroid resistance against *Rhipicephalus* (Boophilus) *microplus* in Telangana, India

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Abstract
Ticks are significant ectoparasites of livestock, acting as vectors of various pathogens and causing major economic losses, especially in tropical regions like India. Due to the lack of alternative control strategies, the extensive reliance on acaricides has resulted in the development of resistance in *Rhipicephalus* (Boophilus) *microplus* populations. This study aimed to assess the resistance status of this tick species against deltamethrin and cypermethrin in six districts of Telangana. The findings provide baseline data essential for developing region-specific tick control strategies. LPT was carried out with 10-12 day old live larvae with same concentrations which were used for adult immersion test. The LC_{50} values of 63.05, 51.18, 118.8, 141.8, 191.5, 35.3 and 139.7, 63.97, 426.9, 391.7, 402.7, 94.6 against Deltamethrin and Cypermethrin were recorded in Rangareddy, Warangal, Mahaboobnagar, Karimnagar, Khammam and Nizamabad districts, respectively. In the present study, out of six field isolates tested with Deltamethrin, two isolates (Warangal, Nizamabad) were resistant at level I and four (Rangareddy, Mahaboobnagar, Karimnagar, Khammam) were resistant at level II. Among the six isolates tested with Cypermethrin, two isolates (Warangal and Nizamabad) were susceptible and four were (Rangareddy, Mahaboobnagar, Karimnagar, Khammam) resistant at level I. The results suggested that the resistance to Cypermethrin was low in Telangana as compared to Deltamethrin.

Keywords: Acaricidal resistance, Telangana, deltamethrin, cypermethrin, larval packet test

Introduction

Ticks are among the most harmful ectoparasites affecting livestock and companion animals worldwide. They also act as carriers for various pathogens, such as bacteria, viruses, and protozoa. Tick infestations can result in severe economic losses in the livestock sector due to decreased productivity, increased veterinary costs, and, in some cases, animal mortality. Their impact is particularly pronounced in tropical and subtropical regions, where favorable environmental conditions support their rapid proliferation and year-round activity. The rapid increase in tick populations and the prevalence of tick-borne diseases are particularly common in certain districts of Telangana (Barman *et al*., 2018) [1]. Among cattle owners in India, the most prevalent method for tick control involves using acaricides particularly synthetic pyrethroids like deltamethrin, flumethrin, and cypermethrin being the most commonly applied agents." (Roy *et al*., 2005, Kumar *et al*., 2013) [2, 3]. If no viable alternative control methods are available, reliance on chemical treatments will likely intensify, thereby increasing the selection pressure on tick populations. In addi-tion, there are no stringent regulations regarding the distribution/purchase of acaricides leading to their injudicious use. Cases of R. *microplus* developing resistance to several classes of acaricides, namely organophosphates (Davey *et al*., 2006) [4], synthetic pyrethroids (Miller *et al*., 2007) [5] and macrocyclic lactones (Martins and Furlong, 2001), are well documented from different parts of the world. "In recent years, multiple investigations have been conducted to evaluate the resistance levels of ticks in India against the acaricides commonly in use (Kumar *et al*., 2011) [6]. In northern India, *R.* (*B.*) *microplus* populations from six distinct agro-climatic zones exhibited variable resistance levels to synthetic pyrethroids like deltamethrin and cypermethrin (Sharma *et al*., 2012) [8]. By contrast, there is a scarcity of research evaluating acaricide resistance in tick populations from southern India.

However, there are no documented reports available to understand the acaricidal resistance status in ticks on the cattle in Telangana state. The current study was undertaken to generate preliminary data on the, resistance status of cattle tick *Rhipicephalus* (*Boophilus*) *microplus* against Deltamethrin, Cypermethrin collected from six districts *viz*., Rangareddy, Mahaboobnagar, Nizamabad, Karimnagar, Khammam and Warangal of Telangana, India by using larval packet test.

Materials and Methods

Telangana, located in southern India on the Deccan Plateau, spans approximately 112,077 km² and lies between 15°46′ - 19°47′ N latitude and 77°16′ - 81°43′ E longitude and is divided into three agro-climatic zones (North, South and Central) based on the range of rainfall received, temperature, type and topography of the soils. Areas with reports of high incidence of tick infestation were selected for ticks collection in the present study.

Technical grade Deltamethrin (99.3%) and cypermethrin (99.5%) (AccuStandard® Inc, U.S.A.) were used for preparation of stock solution in acetone. For the bioassay, a range of working concentrations of acaricides was prepared by diluting the stock solution in distilled water, and these were then tested on field-collected ticks. The resistance status against Deltamethrin and cypermethrin were detected in 10-14-day old unfed tick larvae newly emerged out from the eggs.

The Larval Packet Test was conducted according to FAO (1971) [9] guidelines with minor modifications. The various concentrations utilized in the bioassay of Deltamethrin and cypermethrin were 30, 60, 120, 240, 480 ppm and 50, 100, 200, 400, 800 ppm, respectively. Each concentration was replicated three times and approximately 100-130 larvae were used per replication (n = 100 × 3). Briefly, filter papers measuring 7 × 7 cm (Whatman 541) were impregnated using 0.5 mL of various concentrations of deltamethrin or cypermethrin. These were then dried by incubating them at 37 °C for 30 minutes. The filter papers were folded diagonally to form a triangle and sealed along one edge with adhesive tape, creating an open-ended triangular pouch for placing tick larvae. After insertion of approximately 100 larvae, the open end of each packet was sealed with adhesive tape and the packets were placed in desiccators placed in incubator maintained at 28±1 °C and 85±5% RH. For each concentration of acaricide the test was conducted in triplicate and in control group distilled water was used. The packets were removed after 24 hrs and larval mortality was calculated. The larval mortality was calculated by counting the live and dead larvae from each packet. The concentration at which larval mortality was initiated till the maximum mortality was selected for probit analysis to estimate LC_{50} value.

The regression curve of probit mortality plotted against log values of concentrations was drawn (Finney, 1962) [10] using Graph Pad Prism 4 software.The resistance factor of acaricides for each isolate was estimated by dividing the LC_{50} of each isolate by LC_{50} of reference susceptible IVRI-I line. The LC_{50} value of LPT of Deltamethrin and cypermethrin against susceptible isolate of *R.* (*B.*) *microplus* (IVRI-1 line) were 138.5 and 13.4 ppm, respectively (Sharma *et al*., 2012) [8]. On the basis of RF values, the tick isolateswere classified as susceptible (RF ≤ 1.4), level I-(RF = 1.5-5.0), level II-(RF = 5.1-25.0), level III-(RF = 26-40), and level IV resistant (RF ≥ 41).

Results and Discussion

A progressive increase in the larval mortality was recorded with an increasing concentrations of acaricide with highest mortalities of 100% and 100% with Deltamethrin and Cypermethrin at 480 ppm and 800 ppm, respectively. Various concentrations of synthetic pyrethroids used and mortality percent±SE of larva is presented in Table.1&2. Figure 1 displays the regression graphs of probit mortality plotted against the logarithmic values of progressively increasing concentrations of Deltamethrin and Cypermethrin, respectively.

Table 1: Dose-mortality response of R. (B.) *microplus* collected from six districts against Deltamethrin by Larval packet test.

S. No	Deltamethrin						
		Mean mortality%±SE					
	Concentration	Rangareddy	Warangal	Mahaboobnagar	Karimnagar	Khammam	Nizamabad
1	control	1.8±0.495	3.12±0.73	2.32±0.892	2.1±0.23	2.39±0.76	1.97±0.025
2	30	26.73±0.640	30.58±0.313	7.56±0.466	10±0.57	15.4±1.26	44.1±0.467
3	60	49.12±0.026	58.4±1.01	28.35±0.689	27.33±0.412	28.15±0.456	67.19±1.07
4	120	71.5±0.409	76.1±0.926	54.2±1.08	44.1±0.472	39.04±0.435	81.19±0.76
5	240	84.64±0.549	92.2±1.10	71.33±1.17	62.0±0.99	52.82±0.120	98.13±0.483
6	480	100	100	91.64±1.73	85.4±0. 735	70.4±0.446	100

Table 2: Dose-mortality response of R. (B.) *microplus* collected from six districts against Cypermethrin by Larval packet test

S. No	Cypermethrin						
		Mean mortality%±SE					
	Concentration	Rangareddy	Warangal	Mahaboobnagar	Karimnagar	Khammam	Nizamabad
1	Control	1.52±0.273	1.28±0.319	2.1±0.53	2.57±0.287	0	1.58±0.31
2	50	26.39±0.545	42.01±0.661	0	8.49±0.362	4.56±0.412	31.3±0.709
3	100	39.25±0.469	67.06±1.31	5.7±0.457	19.89±0.769	18.37±0.447	53.1±0.546
4	200	56.22±0.139	79.03±0.708	17.51±1.35	38.27±0.621	31.96±0.273	69.8±1.53
5	400	78.25±0.786	94.53±0.687	47.34±0.70	50.15±0.648	51.34±0.234	86.31±0.678
6	800	100	100	76.65±0.132	65.16±0.477	66.3±0.792	100

The slope of mortality (95% confidence Limit), goodness of fit (R^2), LC_{50} (95% CL), LC_{95} (95% CL) values, resistance factor (RF) and the resistance levels (RLs) in the six district isolates against Deltamethrin and Cypermethrin were presented in Table 3.
In the present study the Lc_{50}values with their resistance factors of field isolates against deltamethrin, cypermethrin when compared with susceptible line detected as 63.05 (5.33), 139.7 (0.57) in Rangareddy, 51.1 (4.3), 63.97 (0.26) in Warangal, 118.8 (10.07), 426.9 (1.76) in Mahaboobnagar, 141.86 (12.02), 391.7 (1.61) in Karimnagar, 191.5 (16.23), 402.7 (1.66) in Khammam and 35.3 (2.99), 94.61 (0.39) in Nizamabad districts. All the four isolates namely Rangareddy, Karimnagar, Khammam and Mahaboobnagar showed level II resistance when compare to other two isolates which showed level I resistance against deltamethrin where as three isolates namely Karimnagar, Khammam and Mahaboobnagar isolates showed level I resistance where as other three isolates showed susceptible status against cypermethrin.

Table 3: Synthetic pyrethroid resistance status in R. (B) *microplus* collected from different districts of, Telangana by LPT

District Isolates	Deltamethrin				Cypermethrin			
	LC_{50} (95% CI)	LC_{95} (95% CI)	RF	RL	LC_{50} (95% CI)	LC_{95} (95% CI)	RR_{50}	RR_{95}
Rangareddy	63.05 (38.23-103.97)	498.91 (302.55-822.7)	5.33	II	139.75 (79.71-245.0)	1618.27 (923.08-2837.01)	0.57	S
Warangal	51.18 (32.10-81.58)	314.96 (197.58-502.08)	4.3	I	63.97 (38.07-107.48)	464.17 (276.2-779.9)	0.26	S
Mahaboobnagar	118.87 (78.55-179.88)	643.71 (425.3-974.0)	10.07	II	426.96 (293.2-621.6)	1848.22 (1269.3-2691.1)	1.76	I
Karimnagar	141.86 (87.67-229.5)	1099.23 (679.3-1778.5)	12.02	II	391.7 (214.14-716.8)	5336.23 (2916.65-9763.0)	1.61	I
Khammam	191.59 (97.0-378.3)	3991.63 (2021.11-7883.3)	16.23	II	402.73 (237.0-684.3)	3742.69 (2202.6-6359.5)	1.66	I
Nizamabad	35.37 (20.03-62.4)	321.65 (182.1-567.9)	2.99	I	94.61 (55.66-160.79)	852.86 (501.8-1449.4)	0.39	S

In the present study, the standard bioassay Larval Packet Test (LPT) recommended by FAO was used for assessing acaricidal resistance to Deltamethrin and Cypermethrin Though there are a number of acaricidal resistance detection methods, bioassay remains the method of choice for susceptibility evaluation of tick populations, based on their toxicological response after exposure to acaricides (Rosario-Cruz *et al*., 2009) [11]. The main purpose of using LPT bioassay was to check the mortality against particular acaricide in ticks on the basis of which the susceptibility or resistance, survival percentage, resistance factor and resistance level were determined. Larval Packet Test (LPT) described by Stone and Haydock (1962) [16] was recommended by FAO, provides repeatable results and selected as the test of choice for surveys. In this test, live larvae are exposed to acaricide impregnated filter paper packets, incubated for specific period of time and then assessed based on larval mortality and is suitable for monitoring resistance where the engorged females were not available. This test takes 5-6 weeks to complete. For conducting bioassays, technical grade (100% pure) Deltamethrin and Cypermethrin were selected over commercial formulations to avoid ambiguity if any in assessing the actual response of active ingredients (Shaw 1966) [12] in acaricides.
In our study there were no susceptible population against Deltamethrin which revealed high to moderate levels of resistance in Telangana and the resistance was more prevalent in (central zone) humid regions than arid (south zone) because the environmental conditions in humid area are very conductive for the development of the ticks. Proportionately the use of acaricides is rapid in humid region with multiple applications subsequently resulting in the development of resistance. This is mainly due to the absence of effective alternative tick control methods that are practically suited to existing farming practices
Similarly, Sharma *et al*. (2012) [8] reported level 1(2.06-4.64) and level II (5.13-9.8) resistance in 10 and 6 regions among the 18 areas surveyed in six agroclimatic regions in India. Singh *et al*. (2014) [13] reported LC_{50} values of 759.6 (5.48) and 302.2 (2.18) in Muktsar and Mansa districts of Punjab which revealed level II and I resistance against Cypermethrin in R. *microplus* where as lower levels of resistance was detected in Hyalomma and they opined that, resistance development is slower in multi host tick when compare to single host tick.
In the present study the lower slope values of field ticks are suggestive of presence of heterogeneous population of ticks in the field having both resistant and susceptible alleles in the populations that allow the presence of heterozygous and homozygous individuals (Castro-Janer *et al*. 2010) [14]. In current study, the results revealed an increased resistance of ticks to Deltamethrin (level I to III) when compare to Cypermethrin (level I to II) may be because of Deltamethrin is one of the most commonly used synthetic pyrethroids for tick control in livestock. Hence, Repeated and widespread use leads to strong selection pressure, promoting resistance development in tick populations. Similar results were reported by Kumar *et al*. (2016) [7] who reported level I to IV resistance against Deltamethrin whereas level I and II resistance by against Cypermethrin in tick isolates collected from Rajastan. Godara *et al*. (2019) [15] found level I to IV resistance in the case of Deltamethrin where as level I and II resistance detected in Cypermethrin. In adition, only one isolate was found with susceptible against Deltamethrin but 3 isolates were found with susceptible status for Cypermethrin.

Conclusion
Acaricidal resistance poses a significant challenge to the effective control of tick populations, especially in regions with intensive chemical use. This highlights the urgent need

for routine monitoring of resistance patterns, adoption of rotational use of acaricides with different modes of action, and implementation of sustainable, non-chemical control measures to mitigate resistance development and preserve the efficacy of existing treatments.

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