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In vitro acaricidal efficacy of deltamethrin, cypermethrin and amitraz against sheep ticks in Karnataka

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Abstract
Acaricide resistance is a major problem in sheep population that hinders the control of the ticks in Karnataka and worldwide. In view of the increasing reports of acaricidal resistance world wide a study was designed to evaluate the efficacy of Amitraz, Deltamethrin and Cypermethrin using larval packet test (LPT) and adult immersion test with differentiating dose (AIT-DD) against different species of ticks. The tick species involved in this study were *Haemaphysalis bispinosa*, *Haemaphysalis intermedia*, *Haemaphysalis kutchensis*, *Hyalomma marginatum issaci*, *Hyalomma anatolicum anatolicum*, *Rhiphicephalus haemaphysaloides* and *Rhipicephalus sanguineus*. In LPT, amitraz at 0.2% induced 100 percent mortality against all species of ticks, whereas cypermethrin and deltamethrin induced 100 percent mortality at 0.3% and 0.4% against all species of ticks. In AIT-DD test amitraz was found to be susceptible at 2.5g/ltr whereas cypermethrin at 0.05g/ltr and deltamethrin at 0.075g/ltr was found to be resistant against all species of ticks in this study.

Keywords: Acaricide resistance, sheep ticks, Karnataka

Introduction
Ticks being major vectors in transmission of tick-borne diseases remain a challenge in livestock in tropical and sub-tropical countries of the world. In tropical country like India, the warm, humid climate favours perpetuation and propagation of ticks. In India, the cost of TTBD control in animals has been estimated to be 498.7 US $ per annum [10]. Ticks are the major constraints to small ruminant production. Control of ticks by chemical acaricides was considered as one of the best feasible methods, but repeated application of these chemicals leads to development of resistance against a range of acaricides [15]. There is a lack of detailed information on acaricidal resistance in Karnataka. Hence, a study was taken up to evaluate the efficacy of commonly used acaricides in control of ticks in sheep flocks in Karnataka by different methods under *in vitro* conditions.

Materials and methods
Collection of adult ticks: Female engorged ticks were collected from different regions of organised farms in Karnataka which were not exposed earlier for any acaricidal treatment. These ticks were used as the standard to assess the susceptibility / resistance status of the ticks collected in the present study. The female engorged field ticks from organised and unorganised sheep farms which were exposed to commonly used acaricides viz., amitraz, cypermethrin and delatmethrin were collected and used as test isolates/ field isolates. Ticks were and identified based on the morphological characters [4, 14]. The collected ticks were washed in tap water and dried on an absorbent paper. After identification of different species of engorged ticks, they were held individually at 28±1 ˚C and 85±5% relative humidity in labelled plastic tubes for oviposition. The eggs laid were separated and allowed to hatch to larvae under similar conditions of incubation in glass tubes closed with cotton plug. Twelve to fifteen day old larvae were taken for evaluation of efficacy of acaricides in by LPT. Larvae were taken from the test tubes by means of a paint brush. One hundred larvae of each tick species along with an control were taken for each concentration of acaricides. They were released on the Whatman No.1 filter paper of 11 cm diameter in plastic petri dishes.

Acaricides: To carry out LPT under *in vitro* conditions commonly used acaricides to control ticks at field conditions were selected viz., amitraz (12.5%), cypermethrin (10%) and delatmethrin (12.5%). The diluent solution was prepared by mixing trichloroethylene and sterile olive oil in ratio 2:1. The different concentrations were made by dilution of appropriate volume of acaricide with diluents to make different concentrations, viz., amitraz (125,250,500,750 and 1000ppm), cypermethrin (100,200,300,400 and 500ppm) and deltamethrin (25, 50, 75,100 and 125ppm).

Larval packet test: The larval packet test (LPT) was done as per FAO guidelines with minor modifications [1]. The different species of ticks larvae of 12-14 days old were treated against different dilutions of acaricides, *viz*., Amitraz (125, 250, 500, 750 and 1000ppm), Cypermethrin (100, 200, 300, 400 and 500ppm) and Deltamethrin (25, 50, 75, 100 and 125ppm) and subjected it for LPT. The packets were removed after 24hrs and larval mortality was calculated. For each concentration of acaricide the test was conducted in triplicate. Then the mortality rate of larvae was calculated by dividing the dead larvae by total larvae of each packet, the larvae which were moving were considered as live. One control packet was prepared for each concentration of acaricide by impregnated it with diluents solution only.

Statistical analysis:
All the data was expressed as mean ±SE. Groups were compared using one way analysis of variance using Graph pad prism 5 software. A value of $P<0.05$ was considered as statistically significant. 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. The data was analyzed by computer program software based on Finney [3].

Resistance levels
The resistance level (RL) in the field population of ticks was classified as susceptible (RF≤1.4), level I (RF=1.5-5), level II (RF=5.1-25), level III (RF=25.1-40) and level IV (RF>40).

Adult immersion test with discriminating dose (AIT-DD)
The adult immersion test with a discriminating dose (AIT-DD) was done as per FAO guidelines [2] to study resistance if present in adult female engorged ticks collected during this study. The selected acaricides were diluted for recommended doses with the help of distilled water. The recommended discriminating dose for AIT-DD test for selected acaricides viz., amitraz: 2.5g/ltr, Deltamethrin: 0.075g/ltr, Cypermethrin: 0.05g/ltr. Ticks immersed in water which laid eggs after 7days were taken as controls. Ticks that were treated with acaricide and still laid eggs were considered resistant. Ticks that were treated with acaricide and did not lay eggs were taken as susceptible. The percentage resistance was calculated as follows: Resistance (%) = (Nt/Nw) × 100.Where Nt= Number of treated ticks laying eggs and Nw= Number of untreated ticks laying eggs.

Results and discussion
The ticks collected in this study were identified as *Haemaphysalis bispinosa*, *Haemaphysalis kutchensis*, *Haemaphysalis intermedia*, *Rhipicephalu sanguineus*, *Rhipicephalus haemaphysaloides*, *Hyalomma anatolicum anatolicum* and *Hyalomma marginatum issaci*. The results of the *in vitro* trials by larval packet test(LPT) on larval stages of different species of ticks i.e at different concentration of Amitraz (0.1%, 0.2%, 0.3%, 0.4%), deltamethrin and cypermethrin (0.05%,0.1%,0.2%, 0.3%) was recorded. After 24hrs of exposure, 100 percent mortality rate was recorded at higher concentrations of deltamethrin (0.4%) and cypermethrin (0.3%), whereas amitraz at lower concentration at 0.2% induced 100 percent mortality (Fig 1-3). The LC_{50} and LC_{95} values of deltamethrin, cypermethrin and amitraz were recorded as presented in Table 2.

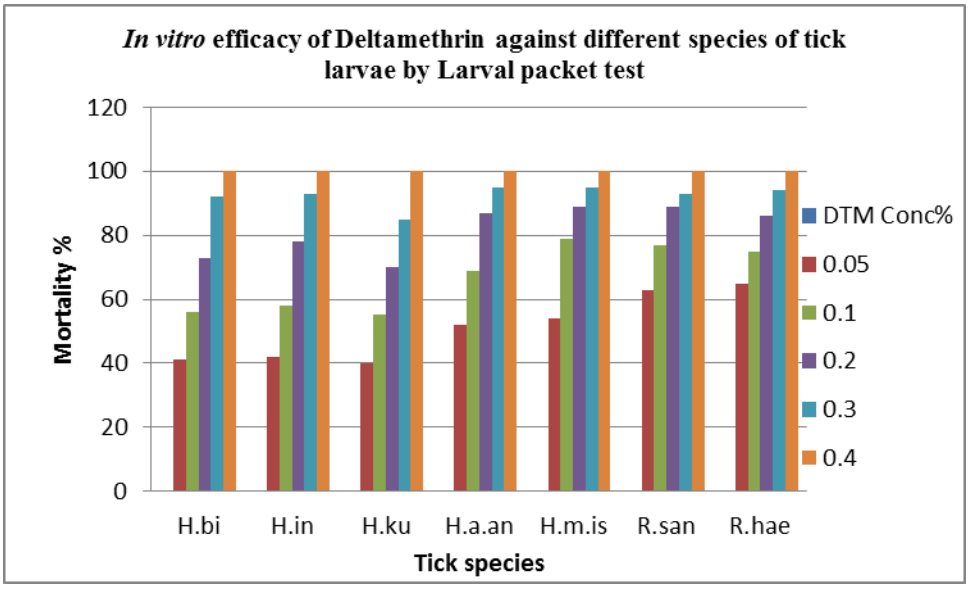

Fig 1: Larval mortality of different tick species H.bi: *Haemaphysalis bispinosa;* H.in: *Haemaphysalis intermedia*; H.ku: *Haemaphysalis kutchensis*; H.a.an: *Hyalomma anatolicum anatolicum*; H.m.is: *Hyalomma marginatum issaci*; R.san: *Rhipicephalus sanguineus*; R.hae: *Rhipicephalus haemaphysaloides* against Deltamethrin (DTM)

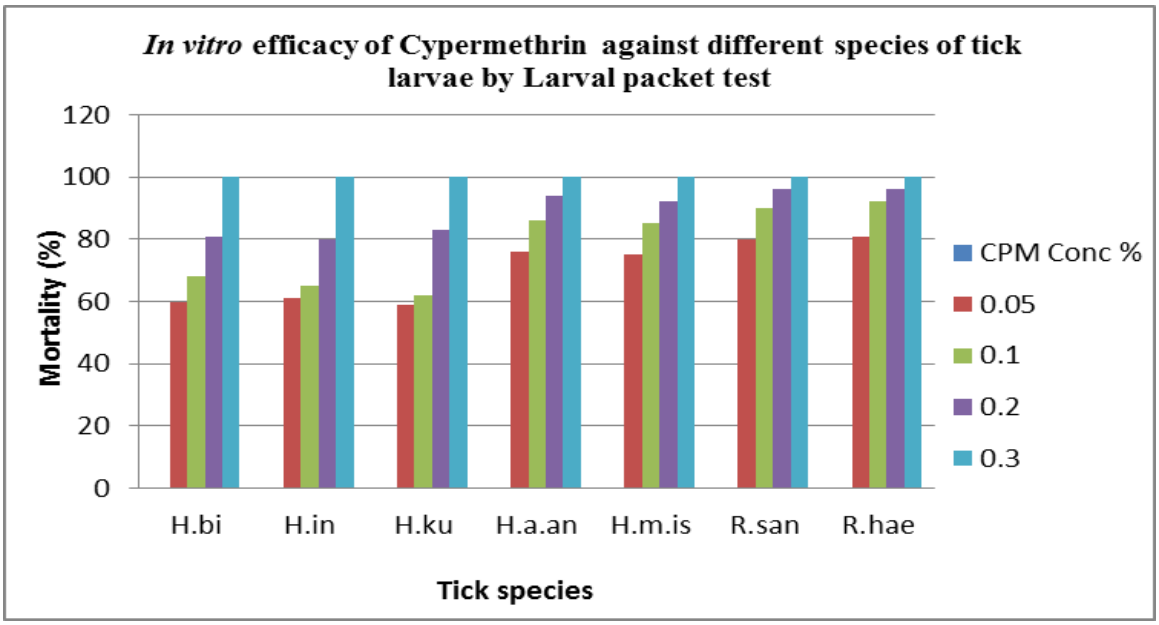

Fig 2: Larval mortality of different tick species H.bi: *Haemaphysalis bispinosa;* H.in: *Haemaphysalis intermedia*; H.ku: *Haemaphysalis kutchensis*; H.a.an: *Hyalomma anatolicum anatolicum*; H.m.is: *Hyalomma marginatum issaci*; R.san: *Rhipicephalus sanguineus*; R.hae: *Rhipicephalus haemaphysaloides* against Cypermethrin (CPM).

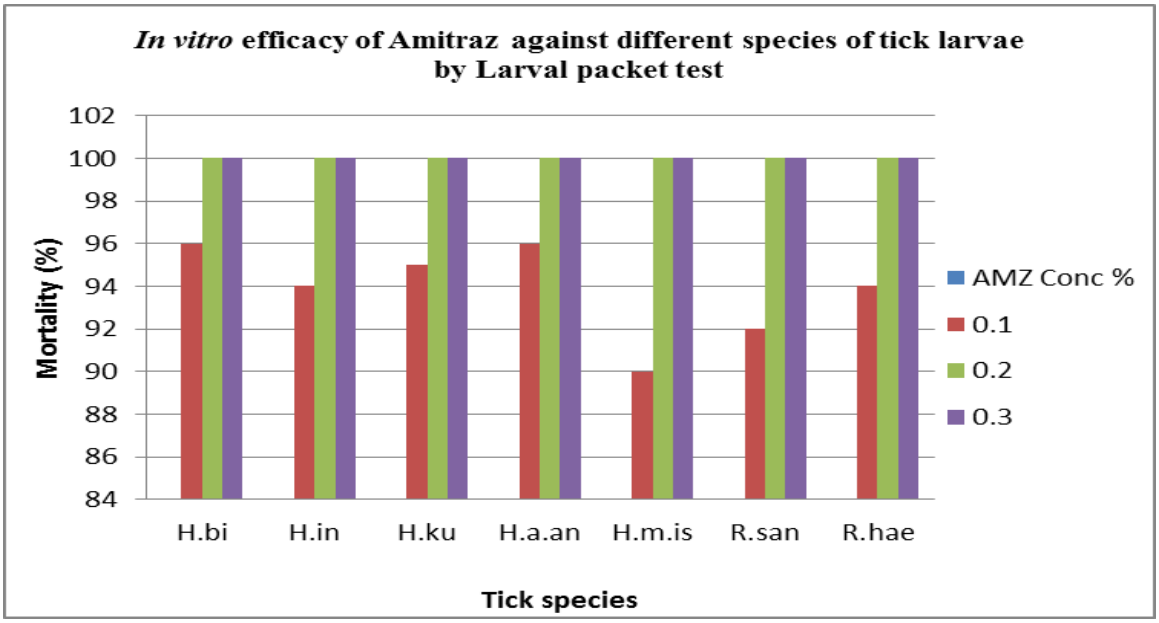

Fig 3: Larval mortality of different tick species H.bi: *Haemaphysalis bispinosa;* H.in: *Haemaphysalis intermedia*; H.ku: *Haemaphysalis kutchensis*; H.a.an: *Hyalomma anatolicum anatolicum*; H.m.is: *Hyalomma marginatum issaci*; R.san: *Rhipicephalus sanguineus*; R.hae: *Rhipicephalus haemaphysaloides* against Amitraz (AMZ)

The results of adult immersion test with discriminating dose for amitraz, deltamethrin and cypermethrin at the discriminating doses of 2.5g/ltr, 0.075 g/ltr, 0.05 g/ltr respectively have also been recorded. The adult immersion test with discriminating doses (AIT-DD) was conducted in triplicates and results were recorded on the average of three replicates against the ticks species *Hyalomma, Haemaphysalis* and *Rhipicephalus* spp. The AIT-DD revealed resistance to cypermethrin and deltamethrin but was found susceptible to amitraz (Table 1)

Table 1: *In vitro* efficacy study of acaricides on engorged female ticks by AIT-DD.

Acaricide	Discriminating doses recomended	Percentage resistance of different species of ticks in this study						
		H.bis	H.int	H.kut	H.a.ana	H.m.issa	R.hae	R.san
Control	-	0	0	0	0	0	0	0
Cypermethrin	0.05g/ltr	20	20	20	30	40	30	20
Deltamethrin	0.075g/ltr	50	60	50	60	60	70	70
Amitraz	2.5g/ltr	0	0	0	0	0	0	0

Note: H.bis: *Haemaphysalis bispinosa;* H.int: *Haemaphysalis intermedia*; H.kut: *Haemaphysalis kutchensis*; H.a.ana: *Hyalomma anatolicum anatolicum*; H.m.issa: *Hyalomma marginatum issaci*; R.san: *Rhipicephalus sanguineus*; R.hae: *Rhipicephalus haemaphysaloides*

Table 2: LC_{50} and LC_{95} fiducial limit, mortality slope, chi square and resistance factor against deltamethrin, cypermethrin and amitraz as determined by LPT against different species of sheep ticks collected from Karnataka, India.

Acaricides	Tick spp	LC_{50}	95%FL	LC_{99}	95%FL	Slope±SE	X2	RF	RL
Deltamethrin	*H. bispinosa*	40.34	4.71-42.21	88.98	51.10- 115.57	2.14±0.47	20.54	8.10	II
	H. kutchensis	42.19	5.14-42.11	89.24	52.68-118.98	2.60±0.56	21.34	7.89	II
	H. intermedia	44.36	5.20- 42.13	89.40	52.20-118.04	2.68±0.56	24.24	2.54	I
	H.a. anatolicum	49.18	5.04-44.64	89.46	51.45- 115.21	2.25±0.51	19.22	6.20	II
	H. marginatum.issaci	48.16	5.02-44.26	89.04	51.21-116.68	2.22±0.49	18.90	8.78	II
	R. haemaphysaloides	59.30	4.04-42.75	85.84	46.36-112.62	2.18±0.51	18.38	7.40	II
	R. sanguineus	62.13	5.23- 44.64	86.34	49.94-114.56	2.20±0.86	18.92	6.09	II
Cypermethrin	*H. bispinosa*	24.24	6.17-48.91	90.13	54.01- 125.70	2.07±0.46	19.63	2.96	I
	H. kutchensis	23.34	4.71-42.12	88.98	51.10-115.57	2.14±0.47	20.54	3.34	I
	H. intermedia	24.18	5.04-44.64	89.46	51.45-115.21	2.25±0.51	19.22	2.45	I
	H.a. anatolicum	29.01	7.20-49.01	92.32	59.45- 123.21	2.20±0.47	21.42	2.36	I
	H.m. issaci	28.16	6.18-46.78	90.46	56.31-119.21	2.01±0.31	19.89	1.89	I
	R. haemaphysaloides	36.73	9.34- 55.67	99.43	102.36-223.20	6.33±0.56	26.92	2.67	I
	R. sanguineus	34.17	5.39-52.90	95.01	97.26-203.24	5.46±0.46	21.95	1.82	I
Amitraz	*H. bispinosa*	1.61	0.85-1.87	2.44	0.24-2.90	1.55±0.31	24.3	1.02	S
	H. kutchensis	1.63	0.64-1.91	2.49	2.26-3.15	1.48±0.33	19.37	1.13	S
	H. intermedia	1.66	1.28-1.85	2.42	2.27-2.68	2.02±0.30	42.95	1.04	S
	H.a. anatolicum	1.90	1.62-2.05	2.53	2.36-2.85	2.02±0.30	42.95	1.07	S
	H. marginatum.issaci	1.86	1.61-2.00	2.48	2.33-2.73	2.05±0.28	53.54	1.15	S
	R. haemaphysaloides	1.70	1.61-1.91	2.37	2.18-2.75	1.92±0.37	27.09	1.14	S
	R. sanguineus	1.71	1.56-1.81	2.28	2.20-2.39	2.23±0.19	33.6	1.04	S

Note: LC_{50}: median lethal concentration; 95% FL: 95% fiducial limit; SE: standard error; X^2 – Chi square; RF- resistant factor; RL- resistance level; S – susceptibility.

Discussion

Results of LPT revealed the development of resistance level I against cypermethrin towards all species of ticks viz., *H. bispinosa, H. kutchensis, H. intermedia, H.a. anatolicum, H. marginatum. issaci, R. haemaphysaloides* and *R. sanguineus* whereas deltamethrin induced resistance level II against all species of ticks except *H. intermedia* inducing resistance level I. Amitraz was found to be susceptible against tick populations of *H. bispinosa, H. kutchensis, H. intermedia, H.a. anatolicum, H. marginatum.issaci, R. haemaphysaloides* and *R. sanguineus.*

Most of the work is reported by different authors in knowing the acaricide resistance against ticks spp *viz.*, *Rhiphicephalus microplus* and *Hyalomma anatolicum anatolicum*. Hence this current study was conducted in knowing the acaricide resistance against different species of ticks viz., *H. bispinosa, H. kutchensis, H. intermedia, H.a. anatolicum, H. marginatum.issaci, R. haemaphysaloides* and *R. sanguineus* in sheep. The susceptibility of amitraz against different species of ticks in this study are with findings of Wharton, 1976; Kemp *et al*. 1998; Martins and Furlong, 2001; and Santos *et al* 2014. The development of cypermethrin and deltamethrin resistance against ticks in this study are with findings of Mavi *et al*. 2007; Shyma *et al*. 2013; Singh *et al*. 2013;Singh *et al*. 2015; Sharma *et al*. 2012; Shyma *et al*. 2012; Kumar *et al*. 2013 and Ghosh *et al*. 2014.

The findings of AIT –DD test in this study showed increased resistance against ticks by deltamethrin followed by cypermethrin, whereas found susceptible to amitraz which are with findings of Pradeep *et al*. 2012 where the author reported 10% resistance by delmethrin and 30% resistance by cypermethrin but found susceptible with amitraz against *Rhiphicephalus microplus* ticks.

The LPT and AIT-DD findings of this study showed increased resistance of ticks when exposed to deltamethrin followed by cypermethrin, whereas ticks were found susceptible to amitraz. The inappropriate repeated useage of deltamethrin and cypermethrin in the regions of this study might be the cause in development of resistance against all species of ticks whereas the minimum useage of amitraz might be the cause to be found susceptible to ticks. Hence the implementations of surveillance systems and preventive programs have to be taken in tropical countries like India for the early detection of resistance. Even farmers should be encouraged to use selective programmes for tick resistance, so that it minimizes the development of tick resistance to acaricides. However, further tests with ticks need to be carried out in order to gain a clear picture of resistant levels for different species of susceptible / resistant ticks from sheep in order to determine the natural variability in the LC_{50}s and LC_{99}s for this test.

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References

1. FAO. Acaricide resistance: Test kit Instructions for use. World Acaricide Resistance Reference Centre, WARCC, Berlin, 1984.
2. FAO. Acaricide resistance test kit. Instructions for use. 2nd Edn, World Acaricide Resistance Reference Centre (WARRC). Berlin. 1999; 11:25-77.
3. Finney DJ. Probit analysis, 3rd edn. Cambridge University Press, Cambridge, UK, 1971.
4. Geevarghese G, Mishra AC. 1st Edn, Elsevier, London. 2011; 1:104-105.
5. Ghosh S, Nagar G. Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research: a review. J Vector Borne Dis. 2014; 51(4):259-70.
6. Kemp DH, Gale RK, Nari A, Sabatini AG. Acaricide resistance in the cattle ticks *Boophilus microplus* and *B. decoloratus*: Review of resistance data; standardization of resistance tests and recommendations for the integrated parasite control to delay resistance. Report to Animal Health service AGAH, FAO. CSIRO Tropical

Agriculture, Long Pocket Laboratories, Queensland, Australia, 1998, 1-33.
7. Kumar SS, Rayulu VC, Rao KS, Kumar NV. Acaricidal resistance in *Rhipicephalus* (*Boophilus*) *Microplus* ticks infesting cattle of Andhra Pradesh. J Entomology and Zoology Studies. 2017; 5(6):580-584.
8. Martins JR, Furlong J. Avermectin resistance of *Boophilus microplus* in Brazil. Vet Rec. 2001; 149(2):64.
9. Mavi PS, Singh NK, Rath SS. Efficacy of closantal, flumethrin and cypermethrin against tick infestation in crossbred calves. Indian Veterinary Journal. 2007; 84:1108-1109.
10. Minjauw B, McLeod A. Tick-Borne Diseases and Poverty. The Impact of Ticks and Tick-Borne Diseases on the Livelihood of Small-Scale and Marginal Livestock Owners in India and Eastern and Southern Africa, DFID Animal Health Programme. Centre for Tropical Veterinay Medicine, University of Edinburgh, UK, 2013; 116p.
11. Pradeep BS, Renukaprasad C, D'souza PE. Evaluation of the commonl used acaricides against different stages of *Rhipicephalus sanguineus* by *in vitro* tests. J Vet. Parasitol. 2010; 24:185-188.
12. Santos TRB, Klafke GM, Pappen FG, Nizoli LQ, Biegelmeyer P, Farias NAR. Comparison of three larval bioassays to evaluate susceptibility of *Rhipicephalus* (*Boophilus*) *microplus* to amitraz Rev. Bras. Parasitol. Vet. Jaboticabal. 2013; 22(4):495-501
13. Sharma AK, Kumar R, Kumar S, Nagar G, Singh NK, Rawat SS, Ghosh S. Deltamethrin and cypermethrin resistance status of *Rhipicephalus* (*Boophilus*) *microplus* collected from six agro-climatic regions of India. Vet Parasitol. 2012; 188(3):337-345
14. Sharrif M. Rec. Indian Museum. 1928; 30:237-284.
15. Shyma KP, Kumar S, Sangwan AK, Sharma AK, Nagar G, Ray D. Acaricide resistance status of *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Haryana. Indian. J. Animal Sciences. 2013; 83(6):591-594.
16. Shyma KP, Kumar S, Sharma AK, Ray DD, Ghosh S. Acaricide resistance status in Indian isolates of *Hyalomma anatolicum*. Exp Applied Acarol. 2012; 58:471-481
17. Singh NK, Jyoti Rath SS. Detection of acaricidal resistance in *Hyalomma anatolicum anatolicum.* India. Vet. J. 2013a; 90:17-19
18. Singh NK, Gelot IS, Jyoti, Singh V, Rath SS. Detection of amitraz resistance in *Rhipicephalus (Boophilus) microplus* from North Gujarat, India. Journal of Parasitic Diseases. 2015; 39(1):49-52.
19. Wharton RH. Acaricide resistance and cattle tick control. Australian Veterinary Journal. 1967; 43:394-398.