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Fenvalerate and diazinon resistance in *Rhipicephalus microplus* ticks collected from North-Western Himalayas, India

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Abstract

The study was conducted to evaluate the resistance status against fenvalerate and diazinon in *Rhipicephalus microplus* ticks collected from five districts of north-western Himalayan region of India using Adult immersion test. Resistance factors (RFs) were calculated between 1.06 to 3.33 for fenvalerate and 2.01 to 3.83 for diazinon. Resistance to fenvalerate was detected at level I in three isolates while two isolates were susceptible. Resistance against diazinon was found at level I in all the isolates examined. Ticks from plains and low altitude areas showed higher RFs wherein intensive animal husbandry practices were followed and the use of chemical acaricides was widespread in comparison to high altitude areas. Data generated on fenvalerate and diazinon resistance status of ticks in north-western Himalayan region will provide new insights in acaricidal resistance particularly from remote areas of this region and will help in formulating suitable control measures.

Keywords: Diazinon, fenvalerate, himalayan region, resistance, *Rhipicephalus microplus*

Introduction

Rhipicephalus microplus is an economically important ixodid tick infesting dairy animals throughout the tropical and sub-tropical regions including Union Territory of Jammu and Kashmir (India) [1]. It is responsible for huge economic losses to dairy farmers through anorexia, blood loss, irritation, udder injury and decrease in milk production and damage to hides. Besides, it transmits babesiosis and anaplasmosis responsible for high mortality in dairy animals [2]. Presently, the most widely used method for the management of tick populations is based on the application of chemical acaricides, onto the animals or in their habitat [3]. However, the use of chemical acaricides as a short term relief measure for quick and cost-effective suppression of tick populations has resulted into development of resistance against acaricides [4]. In order to combat acaricide resistance and develop strategies, there is a dire need of comprehensive data about status of acaricidal resistance across different agro-climatic zones of the country [5]. The mitigation strategies will work only when resistance status of every acaricidal drug in use in every nook and corner of country is evaluated. Previously, resistance against deltamethrin [6, 7], Cypermethrin [4], Malathion and amitraz [8] in *R. microplus* has been reported from north-western Himalayan region of India. However, no published report is available regarding resistance status of fenvalerate and diazinon from this region. Hence, the present study was designed to determine the status of fenvalerate and diazinon resistance in *R. microplus* collected from five districts of north-western Himalayan region of India.

Materials and methods

Study area

Ticks were collected from five districts (Jammu, Samba, Kathua, Poonch and Doda) of Jammu and Kashmir of India. The region is located in north-western Himalayan region which constitutes the Agro Climatic Zone–I of India. It lies between Latitude 32°17’ and 37°05’ N and Longitude 72°31’ and 80°20’ E. The annual temperature and rainfall in these districts ranges from -1 ºC to 46 ºC and 769 mm to 1100 mm, respectively.

Collection of ticks

Ticks were collected from organised and unorganised dairy farms of respective districts. Adult engorged female ticks were collected in vials closed with thin muslin cloth and transported to laboratory. Ticks collected from a particular area (district) were pooled, designated as an isolate, washed thoroughly in water and used for the adult immersion test (AIT) within 24 hours of collection.

Acaricide

Technical grade fenvalerate and diazinon (98.0% pure, Sigma-Aldrich) were used to prepare the stock solutions in methanol. For the experimental bioassay, various dilutions of fenvalerate (100, 200, 400, 800 and 1600 ppm) and diazinon (150, 300, 600, 1200 and 2400 ppm) were prepared from stock formulations using distilled water.

Adult Immersion Test (AIT)

The AIT was performed as described by Sharma *et al*. [9]. Briefly, ticks were immersed in different concentrations of respective drugs for 2 min, dried with filter paper and transferred into the petri dishes. After 24 h, ticks were transferred to plastic tubes covered with muslin cloth and incubated at 28±1°C and relative humidity of 85±5%. The ticks in control group were treated with distilled water. The ticks which did not oviposit even after 14 days were considered as dead [9]. Each concentration was replicated four times with five adult ticks each (n=20) and the following parameters were compared:

(a) Mortality: recorded up to 14 days post treatment.
(b) The egg mass weight laid by the live ticks.
(c) Reproductive index (RI) = egg mass weight/ live tick weight.
(d) Percentage inhibition of oviposition (%IO)=[(RI control-RI treated)/RI control x 100].

Statistical analysis and resistance diagnosis in field isolates

Graph Pad Prism 4 software (La Jolla, CA, USA) was used to generate dose response data by probit method [10]. The resistance factors (RFs) for field tick isolates were calculated as the quotient between LC_{50} of field ticks and LC_{50} of susceptible line of *R. microplus* [11]. The LC_{50} values of diazinon (for adults) against reference acaricides susceptible IVRI-I line of *R. microplus* (LRS) were used [12] while RFs for fenvalerate were worked out considering Doda isolate (Jammu and Kashmir) as susceptible. The lethal concentrations (LC_{50} and LC_{95}) and their respective 95% confidence intervals (CI) were determined by applying regression equation analysis to the probit transformed data of mortality. On the basis of RF, the resistance levels (RL) of *R. microplus* were classified as susceptible (RF<1.4), level I resistance (RF = 1.5-5.0), level II (RF = 5.1-25.0), level III (RF = 25.1-40) and level IV (RF>40) [9].

Results and discussion

The tropical cattle tick, *R. microplus* is predominant in central and north-western parts of India due to the presence of favourable environmental conditions round the year [1, 13]. The excessive use of chemical acaricides for the control of *R. microplus* has resulted into the development of acaricide resistance and resistance in *R. microplus* ticks against various classes of acaricides has been reported from various states of India [4, 8, 9, 14, 15, 16].

As per the recommendations of Jonsson *et al*. [17] and as observed in our previous studies [4, 6, 7, 8], AIT was used in the current study to assess the resistance levels. Technical grade acaricides (fenvalerate and diazinon) were used to nullify the effect of any synergist or proprietary ingredients of commercial acaricide [18]. Besides, the use of a suitable organic solvent also facilitates adsorption of compound over the surface area of target biological materials and possibly enhances penetration of active ingredients of the acaricide across the exoskeleton of the ticks [12].

The data generated from the dose mortality response was utilized to calculate mortality slope, R^2, LC_{50} and LC_{95} values (95% CI), RF and RL which are presented in Table 1. In all the field isolates dose-dependent mortalities were recorded whereas no mortality was recorded in control groups. The regression graphs of probit mortality of *R. microplus* adults plotted against log concentration values of the drugs are presented in Figs. 1 and 2. The mortality slope values ranged from 1.36 to 3.27 with R^2 values of 0.78 to 0.91 for fenvalerate and 2.14 to 3.08 with R^2 values of 0.84 to 0.98 for diazinon. For fenvalerate, the minimum LC_{50} and LC_{95} (95% CI) values of 256.8 (239.6-275.2) and 868.3 (764.2-986.7) ppm were recorded for Doda and Poonch isolates, respectively. Regarding diazinon, the minimum LC_{50} and LC_{95} (95% CI) values of 749.5 (680.8-825.3) and 2833.1 (2473.1-3245.5) ppm were detected for Jammu and Poonch isolates, respectively. The RFs of field isolates against fenvalerate ranged from 1.0 to 3.33 whereas, RFs against diazinon ranged from 2.01 to 3.83. The RL against fenvalerate was found to be at level I in three isolates (Jammu, Samba and Kathua) while two isolates (Doda and Poonch) were found susceptible. The RL against diazinon was found at level I in all the five isolates tested in the current study.

In the present study, higher RFs were found in plains and low altitude areas (Jammu, Samba and Kathua) than high altitude areas (Poonch and Doda). The environmental conditions of the plain area are very conducive for the development of the free living stages of the ticks throughout the most parts of the year leading to heavy tick infestations in the region [1]. Thus, the use of acaricides is rampant in plains with multiple applications per active tick season due to over the counter accessibility of commercial chemical acaricides. The environmental conditions of the high altitude area are unfavourable for propagation and survival of ticks. Generally, low levels of tick infestations on dairy animals are found in high altitude areas which are controlled by non-chemical indigenous methods particularly grooming and hand picking [7, 19].

Table 1: Mortality slope, LC_{50}, LC_{95}, 95% confidence limit (CL), resistance factor (RF), resistance level (RL) values and goodness of fit (R^2) of field isolates of *R. microplus* against fenvalerate and diazinon

Drug	Tick isolate	Slope (95% CL)	R^2	LC_{50} (ppm) (95% CL)	LC_{50} (ppm) (95% CL)	RF[a]	RL
Fenvalerate	Jammu	1.36±0.41 (0.06-2.66)	0.78	857.2 (782.9-938.6)	13680.4 (9952.3-18805.1)	3.33	I
	Samba	2.91±0.55 (1.15-4.66)	0.90	748.8 (697.9-803.5)	2741.5 (2372.6-3167.8)	2.92	I
	Kathua	3.09±0.84 (0.41-5.77)	0.81	642.9 (601.7-687.1)	2179.7 (1893.3-2509.5)	2.51	I
	Poonch	3.27±0.59 (1.38-5.16)	0.91	274.2 (257.5-292.1)	868.3 (764.2-986.7)	1.06	S

	Doda	2.98±0.72 (0.69-5.27)	0.85	256.8 (239.6-275.2)	909.9 (790.5-1047.4)	1.0	S
Diazinon	Jammu	2.14±0.23 (1.39-2.88)	0.96	749.5 (680.8-825.3)	4378.2 (4292.8-4465.4)	2.01	I
	Samba	2.52±0.29 (1.59-3.44)	0.96	1424.2(1312.6-1545.5)	6387.1 (5401.8-7552.1)	3.83	I
	Kathua	2.86±0.39 (1.64-4.08)	0.94	1038.9 (967.1-1116.2)	3881.5 (3349.9-4497.5)	2.79	I
	Poonch	3.08±0.19 (2.47-3.69)	0.98	832.8 (779.1-890.3)	2833.1 (2473.1-3245.5)	2.23	I
	Doda	2.55±0.63 (0.53-4.56)	0.84	850.7 (784.2-922.9)	3748.8 (3176.5-4424.4)	2.28	I
	LRS	7.06±0.54	-	372.0 (341.2-405.2)	635.2 (582.7-692.3)	1.0	S

[a]RF (Sharma *et al*. 2012): LC_{50} of field population/LC_{50} of Laboratory reared susceptible (LRS) for diazinon, LC_{50} of field population/LC_{50} of field susceptible for fenvalerate, Doda*= field susceptible, S = susceptible isolate.

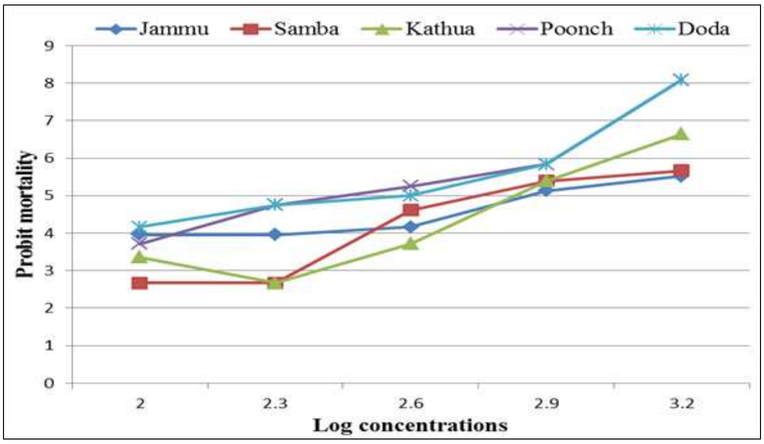

Fig 1: Probit mortality of field isolates of *R. microplus* against log concentrations of fenvalerate

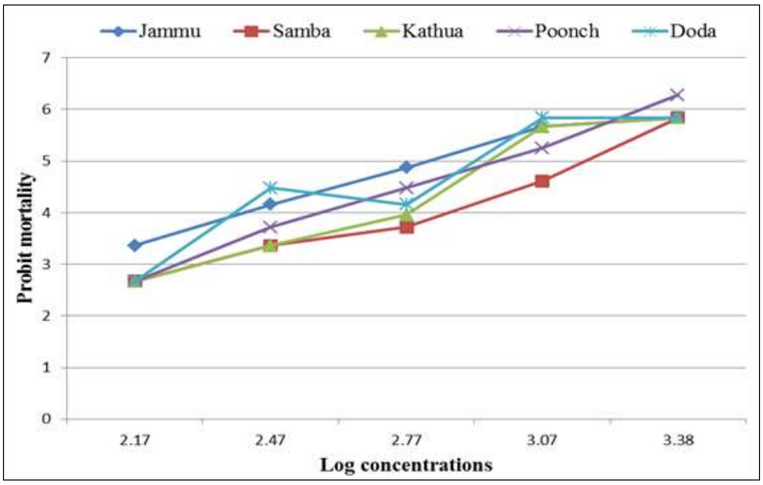

Fig 2: Probit mortality of field isolates of *R. microplus* against log concentrations of diazinon.

Recently, the presence of widespread resistance to diazinon in 20 isolates of *R. microplus* from various agro-climatic regions of India has been reported by Kumar *et al*. [12] with the highest RF of 66.0. Similarly, Chigure *et al*. [20] reported resistance against diazinon in all the four isolates of *R. microplus* collected from two agro-climatic zones of Uttar Pradesh (India) with RFs ranged from 3.5 to 15.07. However, these isolates were found to be susceptible against another OP compound, coumaphos. Shyma *et al*. [21] reported resistance in *R. microplus* against diazinon collected from neighbouring Haryana state (India). Jyoti *et al*. [16] reported presence of fenvalerate resistance in *R. microplus* ticks collected from six districts of central plain zone of Punjab state (India) using larval packet test with RFs ranged from 1.56 to 54.34. The reports on OP resistance have not been reported from the state of Jammu and Kashmir, probably because the use of OP compounds for tick control was stopped by majority of farmers more than fifteen years ago and the use of SPs and formamidine has started to control ticks [7]. The analysis of RFs and RLs data of fenvalerate and diazinon in field isolates revealed that the resistance levels to these acaricides were not alarming. The reduction in egg masses was observed in field isolates exposed to fenvalerate and diazinon as compared to control ticks. The dose response curves for RI were also validated by AIT. The RI of various tick isolates against fenvalerate and diazinon are presented in Figs. 3 and 4, respectively. The RI slope values against fenvalerate ranged from -0.28 to -0.18 with R^2 values of 0.82-0.95 whereas, the RI slope values against diazinon ranged from -0.46 to -0.18 with R^2 values of 0.83-0.95 (Table 2). The slope of RI of ticks exposed to fenvalerate (maximum slope value: -0.28) was comparatively higher than those exposed to diazinon (maximum slope value: -0.46), indicating more susceptible status of *R. microplus* ticks against fenvalerate. The %IO slope values against fenvalerate ranged from 40.8 to 65.4 with R^2 values of 0.79-0.99 (Table 2) whereas, the %IO slope values against diazinon ranged from 50.1 to 71.21 with R^2 values of 0.84-0.95 (Table 2). The slope values of egg mass production by engorged females and RI were negative, showing significant adverse interaction of acaricides with reproductive biology of ticks.

Table 2: Slope (95% CL) of RI and IO (%) and goodness of fit (R^2) of field isolates of *R. microplus* against fenvalerate and diazinon

Drug	Tick isolate	Reproductive index		Percentage inhibition of oviposition	
		Slope (95%CL)	R^2	Slope (95%CL)	R^2
Fenvalerate	Jammu	-0.28±0.04 (-0.39 to -0.16)	0.94	54.20±8.56 (26.95 to 81.45)	0.93
	Samba	-0.24±0.04 (-0.38 to -0.11)	0.91	63.87±11.21 (28.21 to 99.52)	0.91
	Kathua	-0.18±0.05 (-0.34 to -0.03)	0.82	65.40±18.89 (5.30 to 125.5)	0.79
	Poonch	-0.24±0.03 (-0.34 to -0.14)	0.95	49.0±1.98 (42.69 to 55.31)	0.99
	Doda	-0.19±0.02 (-0.27 to -0.12)	0.95	40.80±5.05 (24.74 to 56.86)	0.95
Diazinon	Jammu	-0.18±0.02 (-0.19 to -0.09)	0.95	50.10±5.97 (31.1 to 69.1)	0.95
	Samba	-0.22±0.04 (-0.35 to -0.09)	0.90	54.99±7.88 (29.9 to 80.07)	0.94
	Kathua	-0.21±0.04 (-0.35 to -0.06)	0.87	66.18±15.88 (16.66 to 117.7)	0.85
	Poonch	-0.46±0.05 (-0.64 to -0.27)	0.95	71.21±10.34 (38.30 to 104.1)	0.94
	Doda	-0.41±0.11 (-0.74 to -0.08)	0.83	65.91±16.61 (31.10 to 118.8)	0.84

Conclusion

In conclusion, the results of the current study revealed that *R. microplus* ticks in north western Himalayan region are mildly resistant to fenvalerate and diazinon but the rampant use of these compounds can disturb this equilibrium. Therefore, these acaricides should be used judiciously and the tenets of integrated pest management like biological control, immune response of host and natural resistance should be followed to avert the situation of acaricide resistance in future.

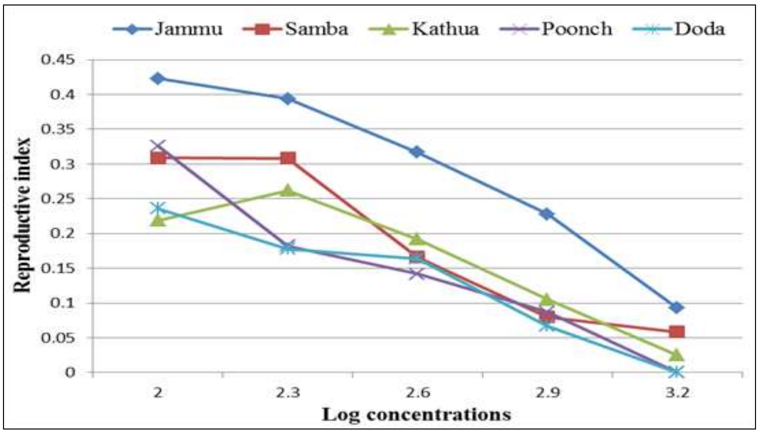

Fig 3: Reproductive index of field isolates of *R. microplus* against log concentrations of fenvalerate

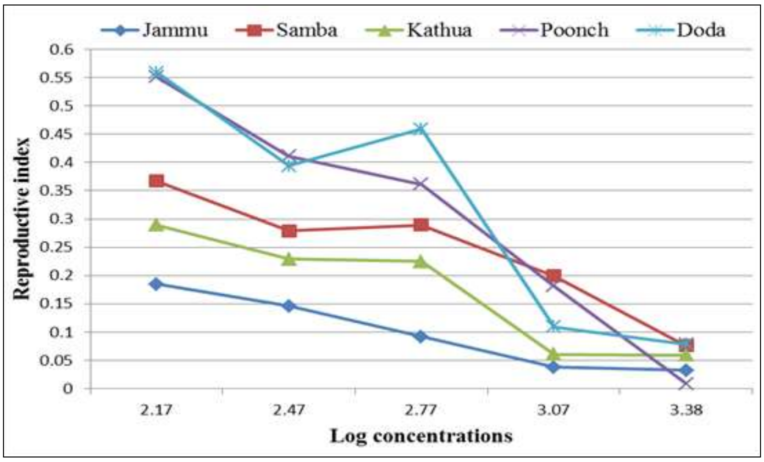

Fig 4: Reproductive index of field isolates of *R. microplus* against log concentrations of diazinon

Conflict of interest

There are no conflicts of interest among the authors.

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