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Esterase mediated pyrethroid resistance in *Rhipicephalus microplus* ticks collected from north-western Himalayas

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Ticks and tick-borne diseases cause huge economic losses to livestock industry. In an agricultural country like India, majority of livestock holders are poor and marginal farmers and loss of 14% of the lactation, as suggested by McLeod and Kristjanson (1999), would pose a serious threat to sustainable livestock dependent system. Tick worry is therefore considered as a major constraint by farmers which are addressed by the hand removal of ticks or repeated application of acaricides every 21–30 days (Ahanger *et al*. 2015). Due to this indiscriminate use of acaricides targeted tick species have developed resistance to multiple acaricides (Fular *et al*. 2018) and the situation is alarming. In order to incorporate integrated pest management, hot spots of acaricidal resistance need to be identified across the country and mitigation strategies need to be developed based on resistance status of that particular agro-climatic zone. Various researchers have deciphered the underlying causes of acaricide resistance which has been ascribed to modifications in the target site, increased metabolism, or inability of the acaricide to penetrate through the outer protective layers of the tick's body and behavioural changes as well (Hernandez 2000, He *et al.* 2002). Insect esterases have been targeted immensely for development of insecticidal resistance since esterases are indispensable to insect metabolism. They are mainly involved in regulation of juvenile hormone levels, digestive processes, reproductive behaviour, functioning of the nervous system and resistance to insecticides (Galego *et al*. 2006). The esterases play an important role in increased metabolic detoxification and target site insensitivity of the drugs, thus contributing to acaricidal resistance (Li *et al*. 2005). Although few published reports are available from India regarding acaricidal resistance to SPs and the involvement of esterases in the development of resistance in *R. microplus* ticks against SPs (Kumar *et al*. 2013, Singh and Rath 2014), no work on this aspect has been carried out from north-western Himalayan region of India. Hence, the present study was undertaken to determine the status of SPs resistance and its underlying detoxification mechanisms in *R. microplus* ticks.

The fully engorged female ticks collected from cattle sheds (eight districts of Jammu region) were thoroughly washed, dried and were kept in separate vials in incubator at 28±1°C and at relative humidity of 85±5%. After 14 days, the female ticks were discarded and their eggs were incubated after closing the glass vials with cotton plugs. About 2–3 weeks old larvae were used to conduct the larval packet test. The larvae were stored at –80°C to conduct biochemical assays. The stock solutions of technical grade deltamethrin and cypermethrin (98.0% pure, Sigma-Aldrich) were prepared after dissolving them in acetone and methanol, respectively. The working concentrations of deltamethrin (12.5, 25, 50, 100 and 200 ppm) and cypermethrin (100, 200, 400, 800 and 1600 ppm) were prepared in distilled water. The larval packet test (LPT) was performed as per described by Sharma *et al*. (2012). Each dose was tested in quadruplets and distilled water was used in the control group. The mortality of larvae was accessed at the end of time period (24 h).

Graph Pad Prism 4 software (La Jolla, CA, USA) was used to analyse dose response data. The LC_{50} values with their respective 95% confidence intervals (CI) were determined by applying regression equation analysis to the probit transformed data of mortality. A value of less than 0.05 was considered significant. The LC_{50} values of reference acaricide susceptible IVRI-I line of *R. microplus* (Sharma *et al*. 2012) were used to calculate the LC_{50} values of deltamethrin and cypermethrin. The resistance levels (RL) were determined as given by Sharma *et al*. (2012).

A microplate enzyme assay was used to determine the esterase activity (μmoles naphthol produced /min/mg protein) with the substrates α- and β-naphthyl acetate in the field tick isolates as described by Singh and Rath (2014). These esterase activities were expressed as enzyme ratio (mean activity of enzyme in field isolate/ mean activity of field susceptible isolate). Total protein concentration of the larval homogenate was determined as given by Bradford (1976).

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The resistance status against deltamethrin and cypermethrin in *R. microplus* ticks are presented in Tables 1 and 2, respectively. In the study region, deltamethrin is more commonly used by farmers to control ticks as it is provided by government to local veterinary hospitals. Hence, the high resistance levels were found against deltamethrin in comparison to cypermethrin. It had been observed that the ticks collected from plain areas (Jammu, Samba and Rajouri) showed higher RFs as compared to low altitude zone (Poonch, Kathua and Udhampur) and high altitude zone (Doda and Kishtwar). The high degree of resistance in plains can be attributed to the indiscriminate use of acaricides over the years (10 to 20 applications per active tick season), wrong dilutions, ease of availability and different brands of this major class of acaricide circulating in the market. On the contrary, ticks collected from higher altitudes were found to be susceptible to the drugs under test. The high susceptibility of SPs at higher altitudes revalidates the fact that availability of drug, managemental practices and frequency of acaricidal application play an important role in development of acaricidal resistance. Since the small and marginal farmers have 2–3 livestock holding capacity and follow age old managemental practice of hand picking the ticks or use of locally available herbal preparations, thus the exposure to chemical acaricides is meagre which conserves the refuge susceptible tick population (Ahanger *et al*. 2015).

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

Tick isolate	Slope (95% CL)	R^2	LC_{50} (ppm) (95% CL)	RF	RL
Jammu	1.80±0.29 (0.88 to 2.72)	0.928	354.3 (345.4-363.5)	30.02	3
Samba	2.09±0.28 (1.19 to 2.99)	0.947	217.9 (213.1-222.7)	18.46	2
Rajouri	3.37±0.82 (0.77 to 5.97)	0.849	92.3 (91.0-93.6)	7.82	2
Poonch	4.09±0.63 (2.08 to 6.1)	0.933	48.0 (47.5-48.5)	4.06	1
Doda	2.68±0.78 (0.19 to 5.16)	0.796	8.4 (7.9-8.4)	0.71	S
Kishtwar	2.52±0.35 (1.41 to 3.63)	0.945	8.5 (8.3-8.6)	0.72	S
Kathua	4.32±0.26 (3.50 to 5.14)	0.989	37.1 (36.8-37.5)	3.14	1
Udhampur	4.19±0.62 (2.22 to 6.16)	0.938	28.9 (28.6-29.3)	2.44	1
LRS*	3.42±0.49	0.871	11.8 (11.6-12.0)	1.00	S

*LRS, Laboratory reared susceptible as per Sharma *et al*. (2012); S, susceptible.

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

Tick isolate	Slope (95% CL)	R^2	LC_{50} (ppm) (95% CL)	RF	RL
Jammu	2.17±0.46 (0.72 to 3.62)	0.883	1823.2 (1784.9-1862.2)	7.52	2
Samba	2.26±0.66 (0.17 to 4.34)	0.797	1780.1 (1744.2-1816.8)	7.34	2
Rajouri	4.76±0.43 (3.38 to 6.14)	0.975	380.0 (376.4-383.7)	1.57	1
Poonch	4.21±0.97 (1.12 to 7.31)	0.862	501.9 (496.5-507.4)	2.07	1
Doda	1.94±0.57 (0.13 to 3.75)	0.794	24.4 (23.8-24.9)	0.10	S
Kishtwar	2.67±0.63 (0.17 to 4.17)	0.798	35.1 (34.3-35.2)	0.14	S
Kathua	4.77±0.66 (2.67 to 6.88)	0.945	264.7 (262.2-267.3)	1.09	S
Udhampur	4.69±0.29 (3.74 to 5.64)	0.988	313.3 (310.2-316.4)	1.29	S
LRS*	10.23±2.46 (241.2-243.6)	0.811	242.4	1.00	S

*LRS, Laboratory reared susceptible as per Sharma *et al*. (2012); S, Susceptible.

Quantitative analysis of general esterase activity revealed a range of 2.466±0.29-8.908±1.68 and 1.279±0.22–5.793±1.24 µmol/min/mg protein for α and β-esterase activity, respectively in tick isolates (Fig. 1). The mean amount of naphthol produced by hydrolysis of α- and β-naphthyl acetate for Samba, Rajouri, Poonch, Kishtwar, Kathua and Udhampur isolates were similar to the susceptible field strain (Doda). Significant elevated levels of both α and β-esterase were detected only in Jammu isolate wherein highest resistance levels of both deltamethrin and cypermethrin were detected. A correlation coefficient (r) of 0.953 and 0.955 was recorded between RFs against deltamethrin and α and β-esterase activities, respectively whereas, the correlation coefficient (r) of 0.874 and 0.872 was recorded between RFs against cypermethrin and á and â-esterase activities, respectively. These results are in accordance with the study carried out in different isolates of *R. microplus* collected from four agro-climatic regions of India (Kumar *et al*. 2013) wherein correlation of determination (R^2) for α- and β-esterase activity was found

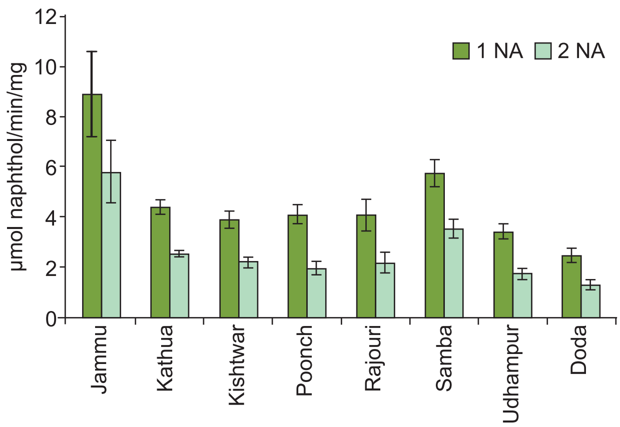

Fig. 1. 1- and 2-napthyl acetate hydrolysis activity (mean±SE) in field isolates of *R. microplus.*

at 73.3% and 55.3% data points of field isolates which are very close to the correlation lines. Similarly, the elevated levels of α- and β-esterases have also been positively correlated in tick isolates of *R. microplus* resistant to both deltamethrin and cypermethrin from Punjab, India (Singh and Rath 2014). Since SPs are esters, they are consequently degraded by esterases. In acaricide resistant tick isolates, pyrethroids could be subjected to enhanced detoxification by cytochrome P450 and carboxylesterases enzymes owing to increased copy number, gene expression or mutations that specifically increase hydrolytic activity (Galego *et al*. 2006).

SUMMARY

Larval packet test (LPT) was used to evaluate resistance status against deltamethrin and cypermethrin in *R. microplus* ticks collected from 8 districts of Jammu region. Resistance against deltamethrin was detected at level 1 in three isolates, level 2 in two isolates and level 3 in one isolate while two isolates were found susceptible. Four isolates were found resistant to cypermethrin whereas, other four isolates were susceptible. Quantitative analysis of general esterase activity revealed a range of 2.466±0.29 to 8.908±1.68 and 1.279±0.22 to 5.793±1.24 μmol/min/mg protein for α- and β-esterase activity, respectively. Multiple pairwise comparisons of the mean values with susceptible field isolate revealed significant elevated levels of both α- and β-esterases in Jammu isolate having highest RFs to both deltamethrin and cypermethrin. It can be concluded that the resistance against SPs has developed in *R. microplus* ticks of north-western Himalayan region, India and esterases play a significant role in conferring the SPs resistance.

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REFERENCES

Ahanger R R, Godara R, Katoch R, Yadav A, Bhutyal A D S, Katoch M, Singh N K and Bader M A. 2015. Deltamethrin resistance in field populations of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) in Jammu and Kashmir state of India. *Experimental and Applied Acarology* **67**: 467–75.

Bradford M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principles of protein dye binding. *Annals of Biochemistry* **72**: 248–54.

Fular A, Sharma A K, Kumar S, Nagar G, Chigure G, Ray D D and Ghosh S. 2018. Establishment of a multi acaricide resistant reference tick (IVRI-V) of *Rhipicephalus microplus*. *Ticks and Tick Borne Diseases* **9**(5): 1184–91.

Galego L G C, Ceron C R and Carareto C M A. 2006. Characterization of esterases in a Brazilian population of *Zaprionus indianus* (Diptera: Drosophilidae). *Genetica* **126**: 89–99.

He H, Chen A C, Davey R B and Ivie G W. 2002. Molecular cloning and nucleotide sequence of a new P450 gene, CYP319A1, from the cattle tick, *Boophilus microplus*. *Insect Biochemistry and Molecular Biology* **32**: 303–09.

Hernandez R, He H, Chen A C, Ivie G W, Waghela S D, George J E and Wagner G G. 2000. Identification of a point mutation in an esterase gene in different populations of the southern cattle tick, *Boophilus microplus*. *Insect Biochemistry and Molecular Biology* **30**: 969–77.

Kumar R, Nagar G, Sharma A K, Kumar S, Ray D D, Chaudhuri P and Ghosh S. 2013. Survey of pyrethroids resistance in Indian isolates of *Rhipicephalus* (*Boophilus*) *microplus*: identification of C190A mutation in the domain II of the para-sodium channel gene. *Acta Tropica* **125**: 237–45.

Li A Y, Pruett J H, Davey R B and George J E. 2005. Toxicological and biochemical characterization of coumaphos resistance in the San Roman strain of *Boophilus microplus* (Acari: Ixodidae). *Pesticide Biochemistry and Physiology* **81**: 145–53.

McLeod R and Kristjanson P. 1999. Tick cost: Economic impact of ticks and TBD to livestock in Africa, Asia and Australia. International Livestock Research Institute (ILRI), Nairobi, Kenya.http://www.esys.com.au and http://www.cgiar.org/ilri.

Sharma A K, Kumar R, Kumar S, Nagar G, Singh N K, Rawat S S, Dhakad M L, Rawat A K S, Ray D D and Ghosh S. 2012. Deltamethrin and cypermethrin resistance status of *Rhipicephalus* (*Boophilus*) *microplus* collected from six agro-climatic regions of India. *Veterinary Parasitology* **188**: 337–45.

Singh N K and Rath S S. 2014. Esterase mediated resistance against synthetic pyrethroids in field populations of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) in Punjab districts of India. *Veterinary Parasitology* **204**: 330–38.