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Research Article

DETECTION OF MULTI-ACARICIDE RESISTANCE IN *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS* (ACARI: IXODIDAE)

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ABSTRACT: Engorged females of *Rhipicephalus* (*Boophilus*) *microplus*(Canestrini 1888) (Acari: Ixodidae) were collected from Jalandhar district, Punjab and acaricide resistance status was assessed by adult immersion test against various class of acaricides *viz*. organophosphates (malathion), synthetic pyrethroids (cypermethrin, deltamethrin and fenvalerate) and formamidines (amitraz). The regression graph of probit mortality of ticks plotted against log values of progressively increasing concentrations of acaricides revealed the slope of mortality and was used for estimation of various lethal concentrations. The LC_{50} (95% CL) and LC_{95} (95% CL) values (ppm) for amitraz [628.4 (592.8 - 666.1) and 1972.7 (1738.1 - 2239.01)], cypermethrin [37.83 (35.02 -40.85) and 183.07 (153.84 - 217.85)], deltamethrin [45.57 (40.32 - 51.49) and 441.56 (342.29 - 569.61)], fenvalerate [142.67 (132.22 - 153.94) and 585.04 (500.46 - 683.91)] and malathion [3436.7 (3251.4 - 3632.6) and 9555.0 (8208.8 - 11122.1)] were estimated. Alow-level resistance status was recorded against formamidines (amitraz), deltamethrin and malathion, whereas, the ticks were found susceptible for cypermethrin and fenvalerate. The dose response curves for egg mass weight, reproductive index and percent inhibition of oviposition were also evaluated for the studied tick population.

Key words: Multi-acaricide resistance, *Rhipicephalus* (*Boophilus*) *microplus*, Punjab.

INTRODUCTION

Rhipicephalus (*Boophilus*) *microplus* (Canestrini 1888) (Acari: Ixodidae), is the major economic threat to the cattle industry in many parts of the world, both directly by physical effects upon infested animals and indirectly through the diseases caused by protozoan parasites transmitted by this tick species. In India, the annual control cost of ticks and tick-borne diseases has been estimated at 498.7 million US$ (Minjauw and Mc Leod 2003). Control of the tick species is largely focused on repeated use of various chemical acaricides *viz*., synthetic pyrethroids (SPs), organophosphates (OPs) and formamidines [Amitraz (Am)] by the cattle owners. However, the indiscriminate and frequent use with improper concentrations has probably contributed to the development of resistance to most of the acaricides in several countries (FAO 2004).

Currently, tick control is more difficult due to the presence of populations resistant to major class of acaricides.There are several reports of resistance development against commonly used chemical acaricides in one host cattle tick from India including Punjab state *viz*. OP (Jyoti *et al.* 2016), SP (Sharma *et al.* 2012, Singh *et al.* 2014a, Nandi *et al.* 2015), Am (Kumar *et al.* 2014, Singh *et al.* 2014b) and ivermectin (Singh *et al.* 2015, Khamgembam *et al.* 2018). However, till date there are no reports of a *R.* (*B.*) *microplus* tick population resistant to the three principal acaricide families (OP, SP and Am) from Punjab and thus, the objective of this study is to document a multi-resistant *R.* (*B.*) *microplus* tick population.

MATERIALS AND METHODS

Acaricides

Technical grade amitraz, cypermethrin, deltamethrin,

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fenvalerate and malathion (Accu Standard® Inc. U.S.A) were used to prepare the stock solution by dissolving in 100% methanol (acetone used for deltamethrin). For the bioassay, various concentrations of the acaricides were prepared in distilled water from the stock solution and tested against the field isolate.

Collection of ticks

Live engorged adult female *R.* (*B.*) *microplus* ticks were collected from shed of dairy farmer reporting acaricide treatment failure (against deltamethrin) from Jalandhar district, Punjab (71°31' E and 30°33' N). Over the last few years, tick control on the farm has been based on the frequent and indiscriminate use of various chemical acaricides. The ticks were transported to the Entomology Laboratory, Department of Veterinary Parasitology, GADVASU, Ludhiana and used for bioassays.

Bioassays

Adult immersion test (AIT) was conducted according to the method of Sharma *et al*. (2012) with minor modifications. Briefly, the engorged females of *R.* (*B.*) *microplus* were immersed in various concentrations of amitraz (62.5, 125, 250, 500 and 1000 ppm), cypermethrin (50, 100, 200, 400 and 800 ppm), deltamethrin (12.5, 25, 50, 100 and 200 ppm), fenvalerate (100, 200, 400, 800 and 1600 ppm) and malathion (1250, 2500, 5000, 10000 and 20000 ppm) for 2 min and then dried on filter paper before transferring into the petri dishes. After 24 h, ticks were weighed and individually transferred to glass tubes covered with muslin cloth and were kept in desiccators placed in incubator maintained at 28±1°C and 85±5% RH. The ticks which did not oviposit even after 14 days post treatment were considered as dead. The control group was treated in similar manner in distilled water. A total of 520 adult engorged female ticks were utilised for the study and each concentration for every drug was replicated twice with ten adults each and the following parameters were recorded:

(a) Mortality: recorded up to 14 days post treatment (dpt).

(b) The egg mass weight laid by the live ticks.

(c) Reproductive Index (RI) = egg mass wt. (EW)/ engorged female wt. (EFW).

(d) Percentage Inhibition of Oviposition (IO%) = [(RI control - RI treated)/RI control × 100)].

Statistical analysis

Dose response data were analyzed by probit method (Finney 1962) using Graph Pad Prism 4 software. This

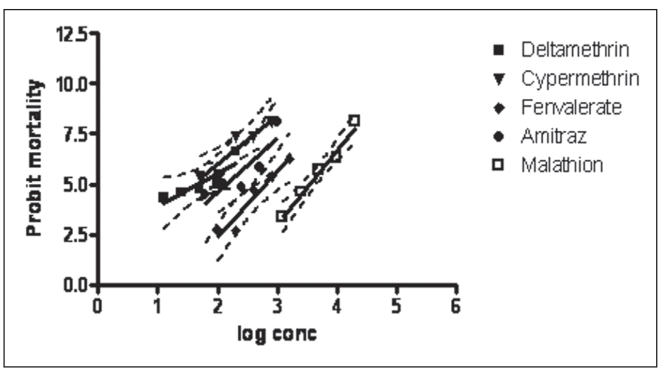

Fig. 1. Dose-mortality curve of *R.* (*B.*) *microplus* against various acaricides.

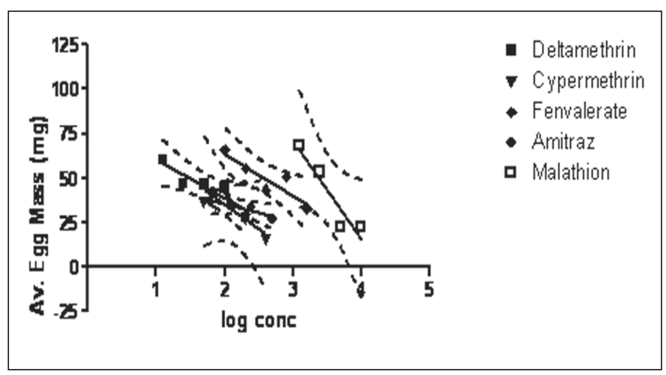

Fig. 2. Dose-Average egg mass weight curve of *R.* (*B.*) *microplus* against various acaricides.

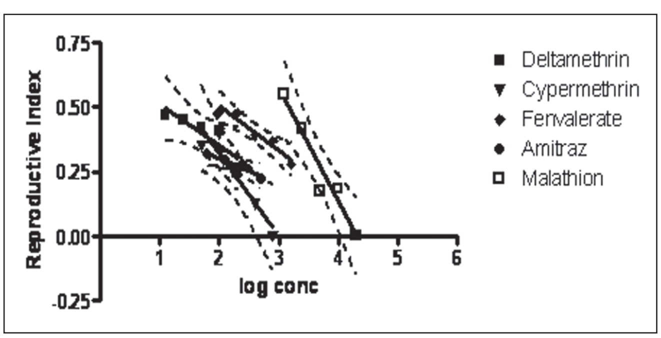

Fig. 3. Dose-reproductive index curve of *R.* (*B.*) *microplus* against various acaricides.

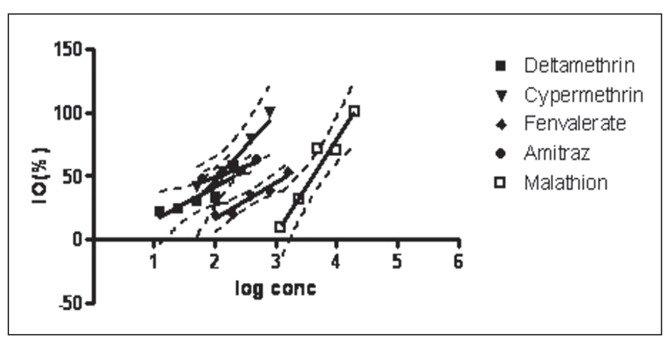

Fig. 4. Dose–inhibition of oviposition (%) curve of *R.* (*B.*) *microplus* against various acaricides.

Table 1. Results of adult immersion test to various acaricides performed on *R.* (*B.*) *microplus*.

Acaricide	Slope ± SE (95% CL)	R^2	LC_{50} (ppm) (95% CL)	LC_{95} (ppm) (95% CL)	$^aRR_{50}$	$^bRR_{95}$
Amitraz	2.67 ± 0.84 (0.004 to 5.35)	0.772	628.4 (592.8-666.1)	1972.7 (1738.1-2239.01)	3.81	4.04
Cypermethrin	2.39 ± 0.48 (0.86 to 3.92)	0.891	37.83 (35.02-40.85)	183.07 (153.84-217.85)	0.27	0.52
Deltamethrin	1.66 ± 0.54 (-0.08 to 3.41)	0.753	45.57 (40.32-51.49)	441.56 (342.29-569.61)	3.40	14.92
Fenvalerate	3.30 ± 0.52 (1.64 to 4.95)	0.930	142.67 (132.22-153.94)	585.04 (500.46-683.91)	0.12	0.26
Malathion	3.69 ± 0.31 (2.67 to 4.71)	0.978	3436.7 (3251.4-3632.6)	9555.0 (8208.8-11122.1)	1.24	1.86

$^aRR_{50}$: LC_{50} of tick stain/LC_{50} of susceptible strain. $^bRR_{95}$: LC_{95} of tick strain/LC_{95} of susceptible strain.

Table 2. Reproductive index and inhibition of oviposition (%) of *R.* (*B.*) *microplus* against various acaricides.

Acaricide	Reproductive Index (RI)			Inhibition of Oviposition (IO%)		
	Slope (95% CL)	R^2	p value	Slope (95% CL)	R^2	p value
Amitraz	-0.099 ± 0.02 (-0.18 to -0.01)	0.924	0.038	16.53 ± 3.33 (2.16 to 30.89)	0.924	0.038
Cypermethrin	-0.323 ± 0.07 (-0.54 to -0.09)	0.874	0.019	53.77 ± 11.80 (16.22 to 91.31)	0.874	0.019
Deltamethrin	-0.161 ± 0.05 (-0.32 to 0.01)	0.756	0.055	26.73 ± 8.76 (-1.14 to 54.61)	0.756	0.055
Fenvalerate	-0.171 ± 0.03 (-0.25 to -0.09)	0.932	0.008	28.41 ± 4.42 (14.34 to 42.48)	0.932	0.008
Malathion	-0.441 ± 0.06 (-0.65 to -0.23)	0.939	0.007	73.37 ± 10.79 (39.05 to 107.7)	0.939	0.007

analysis included probit transformation of percentage mortality and natural logarithm transformation of concentration. The lethal concentrations at 50% (LC_{50}) and 95% (LC_{95}) with 95% confidence limits (CL) were estimated. Resistance ratios (RRs) were worked out by the quotient between LC_{50}/LC_{95} of field isolates and LC_{50}/LC_{95} of susceptible isolate (Singh *et al.* 2015). The LC values of susceptible IVRI-I line of *R.* (*B.*) *microplus* of various acaricides were adopted as per Sharma *et al.* 2012 (cypermethrin and deltamethrin), Kumar *et al.* (2014) (amitraz) and Kumar *et al.*(2015) (fenvalerate and malathion).

RESULTS AND DISCUSSION

The *R.* (*B.*) *microplus* population in this study showed resistance to all acaricides evaluated except cypermethrin and fenvalerate. The tested population showed different resistance ratios to various acaricides and highest RR_{95} of 14.92 was recorded against deltamethrin.The slope, value of goodness of fit (R^2), LC_{50}, LC_{95}, resistance ratios (RRs) to various acaricides and their respective 95% CL in the tested population are shown in Table 1. The regression graph of probit mortality of ticks plotted against log values of progressively increasing concentrations of acaricidesis shown in Fig. 1. The dotted lines in the regression curve represented the 95% confidence limits.

The dose response curves for egg mass weight, reproductive index (RI) and percentage inhibition of oviposition (IO%) of *R.* (*B.*) *microplus* were also studied by AIT. The egg mass weight of ticks treated with different concentrations of acaricides decreased with increasing concentrations of drug and the slope (95% CL) of egg mass was negative (Fig. 2).The slope (95% CL) of RI of ticks treated with different concentrations of acaricides was also negative (Fig. 3). Further, there was an increase in the IO% in ticks with increase in drug concentration and thus a positive slope (95% CL) was recorded (Fig. 4). Results showed significant decrease ($p<0.05$) in the efficiency of survived treated ticks to convert the live weight into egg mass except for deltamethrin ($p>0.05$) thus establishing the marked effect of the acaricides on the RI of treated ticks (Table 2). Also, there was a significant increase ($p<0.05$) in the IO% in ticks with increase in drug concentration for all acaricides (except for deltamethrin) (Table 2).

This study is the first report of *R.* (*B.*) *microplus* tick population resistant to three families of chemical acaricides commonly being used in Punjab. Among the various acaricides used for the control of ticks in livestock, resistance has been reported against OP (Jyoti *et al.* 2016), SP (Sharma *et al.* 2012, Singh *et al.* 2014a, Nandi *et al.* 2015), amitraz (Kumar *et al.* 2014, Singh *et al.* 2014b) and ivermectin (Singh *et al.* 2015, Khamgembam *et al.* 2018). Reports of multi-resistance against various acaricide groups are available against *R.* (*B.) microplus* ticks from various parts of world (Rodriguez-Vivas *et al.* 2006, Mendes *et al.* 2011, Fernandez-Salas *et al.* 2012), but are scanty from India (Shyma *et al.* 2013).

The information obtained about the acaricide usage in cattle-tick control, specifically indiscriminate choice of the acaricide, frequent usage of spray formulations, high frequency of treatments and random substitution of acaricide groups reveals a favorable scenario for the rapid development of multiple drug resistance in the area. The development of resistance in a tick population is due to factors related with acaricide use, ecological niches and the genus of ticks involved (Kunz and Kemp 1994). During the last decades, the tick population utilized in the study has been subjected to frequent applications of OP, SP and amitraz which has led to selection of multi-resistant individuals.

The standard bioassay recommended by FAO for testing resistance to acaricides in *R.* (*B.*) *microplus* is the larval packet test (LPT) originally described by Stone and Haydock (1962). The LPT takes 5–6 weeks to complete, is a laborious test and requires significant laboratory resources to conduct the test routinely. Whereas, AIT can be conducted in ease and data can be generated within two weeks time (Castro-Janer *et al.* 2009).The AIT uses engorged females which are immersed in acaricides and are based on rate of oviposition between females of two groups, treated and control. The eggs are analyzed by weight and direct mortality can be taken into consideration by comparing females that oviposit or not. AIT has been utilized by various workers for estimation of efficacy of various acaricides against *R.* (*B.*) *microplus* (Sharma *et al.* 2012, Singh and Rath 2014).

For conducting the bioassay, technical grade acaricides was selected over commercial formulation as commercial products are prepared with many proprietary ingredients and it is difficult to assess the responses due to active ingredients (Shaw 1966). The stock solutions were prepared by dissolving in 100% methanol and the working concentrations were prepared with distilled water. Use of organic solvent facilitates adsorption of compound over the surface area of target biological materials and also enhances penetration of active ingredients of the acaricide across the exoskeleton (Sharma *et al.* 2012).

CONCLUSION

The present study reports for the first time a *R.* (*B.*) *microplus* population in Punjab with different levels of resistance to SP, OP and amitraz. The uncontrolled use of these products encompassing all of the three most used classes of acaricides available on the market in the study area may promote more incidences of multiple drug resistance. This information is fundamental, in order to establish the monitoring of resistance and contributing to the rational use of acaricides for the control of *R.* (*B.*) *microplus*. The results signify that there is a need for continuous monitoring of acaricide resistance in field situation for strategic application of available acaricides and for maintaining the life span of the product.

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