Fenvalerate resistance status in *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) from Punjab, India

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Abstract Larval packet test was used for evaluating the resistance levels in *Rhipicephalus (Boophilus) microplus* collected from different districts of central plain zone of Punjab state, India against fenvalerate. The regression graphs of probit mortality of larvae plotted against log values of progressively increasing concentrations of fenvalerate were utilized for the estimation of lethal concentration for 50 % (LC₅₀) and 95 % (LC₉₅) values against various field isolates of *R. (B.) microplus*. The slope of mortality (95 % confidence levels) varied from 0.730 ± 0.097 (0.419–1.043) to 1.455 ± 0.281 (0.558–2.352) and the value of R^2 varied from 0.881 to 0.997. From the regression equation the values of LC₅₀ and LC₉₅ were recorded in range of 184.39–1,338.01 and 3,253.33–112,706.26 ppm, respectively. Among the various tick isolates resistance factors in range of 1.56–54.34 were determined and all field isolates studied were found resistant against fenvalerate. Two field isolates (Jalandhar and Ludhiana) showed level I resistance; three (Patiala, Fatehgarh Sahib and Amritsar) showed level II and Kapurthala isolate showed level IV resistance. The data generated on fenvalerate resistant status will help in judicious use of the drug and formulation of effective tick control strategy for the region.

Keywords Acaricide resistance · Fenvalerate · LPT · Punjab · *Rhipicephalus (Boophilus) microplus*

Introduction

Ticks are important blood sucking parasites, causing deleterious effects primarily in two ways, firstly, they suck significant quantities of blood which itself affect the weight gain and milk production of dairy animals and secondly, the impact of tick borne diseases is immense on livestock health (Graf et al. 2004). Amongst the different species of ticks, *Rhipicephalus (Boophilus) microplus* is a major one affecting livestock health in Punjab state, as a debilitating agent itself and as a vector of babesiosis and anaplasmosis (Singh and Rath 2013). On global basis the losses incurred by livestock industry due to ticks and tick borne diseases (TTBDs) was estimated in the range of 14,000 to 18,000 million US\$/year (De Castro 1997). In India, the annual cost of control of TTBDs of cattle has been estimated as US\$498.7 million (Minjauw and McLeod 2003).

Presently, control of *R. (B.) microplus* is largely focused on repeated use of commercial acaricides and historically, this proved to be an effective strategy for mitigating the tick's economic impact on the livestock industry. However, application of these chemical acaricides is often accompanied by serious drawbacks, including the development of acaricide resistant ticks, environmental contamination, and contamination of milk and meat products with insecticide residues (Graf et al. 2004). A survey based on questionnaires in a sampled population of manufacturers and farmers reported the presence of a wide spread acaricidal resistance in India (FAO 2004). Among the various acaricides, synthetic pyrethroids (SPs) are most common commercially available acaricides in India and at present are

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being predominantly used for tick control (Sharma et al. 2012). The indiscriminate and incessant use of SPs with improper concentrations has probably contributed to the development of resistance. Data on tick resistance to SPs particularly cypermethrin and deltamethrin is available from India (Vatsya and Yadav 2011; Sharma et al. 2012; Khajuria et al. 2014) including Punjab state (Singh et al. 2010, 2012, 2014a). However, reports on resistance to fenvalerate, a third generation SP are scanty from India (Abdullah et al. 2013; Ravindran et al. 2014) and no reports are available from Punjab state, in particular. Therefore, the current study was undertaken to detect fenvalerate resistance in various field isolates of *R. (B.) microplus* collected from different districts of central plain zone, Punjab (India).

Materials and methods

Study area

The study was undertaken in six districts (Amritsar, Fatehgarh Sahib, Jalandhar, Kapurthala, Ludhiana and Patiala) of central plain zone of Punjab state, India. The average annual rainfall of this region varies from 500 to 800 mm and climatic conditions are moderately humid. Such prevailing environmental conditions prove to be ideal for both the survival and propagation of ticks.

Collection of ticks

The live engorged adult female ticks were collected from dairy sheds harbouring cross bred cattle and buffaloes during April to July, 2013. Further data related to the frequency, type and mode of acaricide usage adopted by the livestock owners along with their experiences about efficacy of the commonly used acaricides were recorded. The ticks were collected in vials, closed with muslin cloth for air and moisture exchange, brought to the Entomology Laboratory, Department of Veterinary Parasitology, GADVASU, Ludhiana, were cleaned and identified under stereo-microscopic (Sen and Fletcher 1962), labelled and kept at 28 ± 1 °C and 85 ± 5 % relative humidity for egg laying. The eggs laid were further maintained in similar conditions for optimal hatching and 10–14 days old unfed larvae were utilized for the conduction of bioassay for resistance detection against fenvalerate.

Acaricide

Technical grade fenvalerate 100 % pure (AccuStandard® Inc. U.S.A) was used in the present study and the stock solution was prepared in methanol. For the bioassay,

various concentrations of fenvalerate were prepared in distilled water from the stock solution and tested against different collected field isolates of *R. (B.) microplus*.

Larval packet test (LPT)

The LPT was conducted as per the FAO (1971) guidelines with minor modifications. In brief, 0.5 ml each of different concentrations of fenvalerate (100, 200, 400, 800 and 1,600 ppm) in water were used to impregnate 7.0 cm × 7.0 cm filter paper (541 Whatman). The aqueous solution of acaricide was dried by keeping the filter paper in an incubator at 37 °C for 30 min. The filter papers were then folded half diagonally and sealed on one side with an adhesive tape, forming an open-ended triangular packet to place tick larvae. After placing approximately 100 larvae, the open end of each packet was sealed with adhesive tape, kept in a desiccator and placed in an incubator to maintain 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 h, and larval mortality was calculated by counting the total live and dead larvae from the each packet.

Estimation of resistance status

Dose response data were analyzed by probit method (Finney 1962) using GraphPad Prism 4 software. The lethal concentration for 50 % (LC₅₀) and 95 % (LC₉₅) values of fenvalerate against various field isolates of *R. (B.) microplus* were determined by applying regression equation analysis to the probit transformed data of mortality. Resistance factors (RF) were worked out by the quotient between LC₉₅ of field tick isolates and LC₉₅ of reference acaricides susceptible IVRI-I line of *R. (B.) microplus*. On the basis of RF, the resistance status was classified as susceptible (RF < 1.4), level I (RF = 1.5–5.0), level II (RF = 5.1–25.0), level III (RF = 25.1–40) and level IV (RF > 40.1) (Sharma et al. 2012).

Results and discussion

The predominant tick species recorded in dairy animals comprising of cross bred cattle and buffaloes was *R. (B.) microplus* from the study region as previously reported by Singh and Rath (2013). Farmers from this area were reported to have high dependence on chemical acaricides particularly SPs and in most cases were being used without maintaining optimum concentrations leading to their inefficacy against tick control.

The slope of mortality (95 % confidence levels), goodness of fit (R^2), LC₅₀ (95 % CL), LC₉₅ (95 % CL) values,

RF of fenvalerate and the resistance levels (RL) in the field isolates are presented in Table 1. The progressive increase of concentration of fenvalerate caused an increase in mortality of larvae of various field isolates of *R. (B.) microplus* whereas in control group the maximum percent mortality of 1.79 was recorded. The regression graph of probit mortality of various field isolate larvae plotted against log values of progressively increasing concentrations of fenvalerate is shown in Fig. 1. The dotted lines in the regression curve represented the 95 % confidence limits. The slope of mortality (95 % CL) varied from 0.730 ± 0.097 (0.419–1.043) to 1.455 ± 0.281 (0.558–2.352) and the value of R^2 varied from 0.881 to 0.997 among various field isolates. From the regression equation the values of LC_{50} (95 % CL) and LC_{95} (95 % CL) were recorded in range of 184.39 (171.5–198.2) to 1338.01 (1290.2–1387.5) and 3253.33 (2771.1–3819.4) to 112706.26 (97160.5–130739.2) ppm, respectively. The RFs were in range of 1.56–54.34 and all field isolates studied were found resistant against fenvalerate. Among the six field isolates two isolates (Jalandhar and Ludhiana) showed level I resistance; three isolates (Patiala, Fatehgarh Sahib and Amritsar) showed level II and one isolate (Kapurthala) showed level IV resistance.

For detection of acaricide resistance in ticks various bioassay techniques have been developed worldwide, however, the most widely used method for several acaricides is LPT initially designed by Stone and Haydock (1962). This method has also been adopted by the FAO as a standard technique for acaricide resistance detection and measurement (Jonsson et al. 2007). In LPT, live larvae are exposed to filter paper packets impregnated with acaricide, incubated for acaricide-specific time periods and then larval mortality is determined. The LPT has been used to detect resistance against various chemical acaricides viz. organochlorines, organophosphates, SPs, amidines and macrocyclic lactones, in single and multi-host ticks. It is a repeatable test that performs better than the widely used adult immersion test (Jonsson et al. 2007).

Further, in the present study, technical grade fenvalerate was selected over commercial formulation for the bioassay

because commercial products are prepared with many proprietary ingredients and it is difficult to assess the responses due to active ingredients (Shaw 1966). For the preparation of stock solution technical grade fenvalerate was dissolved in 100 % methanol and the working concentrations were prepared using water. The use of organic solvents facilitates the adsorption of compound over the surface area of target biological materials and possibly enhances penetration of active ingredients of the acaricide across the exoskeleton (Sharma et al. 2012). The LC_{50} and LC_{95} values and confidence limits of an acaricide provide a clue about the resistance status of the tick population of a particular area (Vieira-Bressan et al. 1999). These values, however, provide no information about the number of resistant and susceptible individuals in the population and also do not give any clue about the factors involved in resistance development but these values are helpful for documentation of the existence and level of resistance in the population (Abdullah et al. 2013).

The tick population has immense potential for rapidly developing resistance due to their biological and behavioural characteristics and resistance to different active ingredients has been reported in almost all countries where this parasite occurs (Alonso-Diaz et al. 2006). A survey based on questionnaires in a sampled population of manufacturers and farmers reported the presence of a wide spread acaricidal resistance in India (FAO 2004). Among the various acaricides used in Punjab for the control of ticks in livestock, resistance has been reported against most of the acaricides in *R. (B.) microplus* viz. organophosphate compounds (Jyoti et al. 2013), SPs particularly cypermethrin and deltamethrin (Singh et al. 2010, 2012, 2014a) and amitraz (Singh et al. 2014b). However, till date no reports on resistance to fenvalerate, a third generation SP are available from the region. Recently, resistance to fenvalerate in *R. (B.) microplus* collected from Uttarakhand (Abdullah et al. 2013) and *R. (B.) annulatus* from Kerala (Ravindran et al. 2014) had been reported.

Resistance to SPs is usually described in terms of family resistance and ticks resistant to one pyrethroid compound

Table 1 The results of larval packet test to fenvalerate performed on various field isolates of *R. (B.) microplus*

Tick isolate	Slope (95 % CL)	R^2	LC_{50} (ppm) (95 % CL)	LC_{95} (ppm) (95 % CL)	RF	RL
Amritsar	1.163 ± 0.171 (0.616–1.709)	0.938	656.98 (627.6–687.6)	16,940.08 (16,926.8–16,953.3)	8.16	II
Fatehgarh Sahib	1.455 ± 0.281 (0.558–2.352)	0.898	1,338.01 (1,290.2–1,387.5)	17,931.15 (16,618.3–19,347.7)	8.65	II
Jalandhar	0.730 ± 0.097 (0.419–1.043)	0.948	184.39 (171.5–198.2)	3,253.33 (2,771.1–3,819.4)	1.56	I
Kapurthala	0.770 ± 0.163 (0.251–1.290)	0.881	835.75 (781.1–894.2)	112,706.26 (97,160.5–130,739.2)	54.34	IV
Ludhiana	1.329 ± 0.150 (0.850–1.807)	0.963	292.99 (281.7–304.7)	5,032.77 (4,625.7–5,475.6)	2.42	I
Patiala	1.412 ± 0.040 (1.283–1.541)	0.997	1,161.86 (1,119.3–1,206.0)	16,851.12 (15,574.0–18,232.9)	8.12	II
Susceptible	6.83 ± 0.54	0.950	1,193.5 (1,170.9–1,218.2)	2,074.0 (1,995.3–2,158.1)	1.0	–

RF Resistance factor; RL Resistance level

Fig. 1 Dose mortality curve of different isolates of *R. (B.) microplus* against fenvalerate (A Amritsar; B Fatehgarh Sahib; C Jalandhar; D Kapurthala; E Ludhiana; F Patiala)

cannot be controlled by another compound from the same family for a long time (Millar 1988). The resistance recorded in most of the field isolates of *R. (B.) microplus* studied from Punjab state, India is relatively less pronounced to fenvalerate (except Kapurthala isolate) but it is very likely that higher resistance will be acquired by these ticks in near future if use of SPs is continued. To reduce the utilization of these chemical acaricides with reduced efficacies other alternative approaches need to be explored involving the use of eco-friendly sustainable methods in a strategic integrated manner.

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Conflict of interest Nil.

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