Original Article

Detection of Malathion Resistance in *Hyalomma anatolicum anatolicum* from Bathinda District, Punjab

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ABSTRACT

Objective: The resistance status against malathion in *Hyalomma anatolicum anatolicum* ticks collected from Bathinda district, Punjab, was evaluated by adult immersion test (AIT). **Materials and Methods:** Technical grade malathion was used for conduction of AIT with 2 min immersion time protocol. The regression graph of probit mortality of ticks was plotted against log values of increasing concentrations of malathion and was utilized for the determination of slope of mortality, LC_{50}, LC_{95} (95% confidence interval [CI]) and resistance factor (RF). The reproductive parameters of treated ticks viz. egg mass weight, reproductive index (RI), and percentage inhibition of oviposition (% IO) were also studied. **Results:** The increasing concentration of malathion showed an upward trend in per cent tick mortality. The slope of mortality (95% CI) was 2.489 ± 0.719 (2.489 ± 0.719) and value of goodness of fit (R^2) was 0.799. The LC_{50} (95% CI) and LC_{95} (95% CI) values were recorded as 9099.2 (8378.6–9881.7) and 41,511.3 (35,060.2–49,149.4) ppm, respectively, with RF as 16.60 indicating level II resistance status. A negative dose-dependent slope of egg mass weight (-91.79 ± 25.15 [-171.8 to -11.76]) was recorded as the survived ticks laid significantly ($P = 0.0355$) fewer eggs. The mean RI of treated ticks decreased with increasing concentrations of drug and the slope (95% CI) was -0.293 ± 0.059 (-0.482 to -0.105). Further, a dose-dependent significant increase ($P = 0.0157$) in the mean % IO was recorded in treated ticks. **Conclusions:** The results of the current study would be useful in the formulation and implementation of effective tick strategies in the region.

Key words: *Hyalomma anatolicum anatolicum*, malathion, Punjab, resistance

INTRODUCTION

Hyalomma anatolicum anatolicum, a multi-host tick, is widely distributed and considered as an economically important ixodid ticks infesting dairy animals in India,[1] particularly Punjab state.[2] The bite marks inflicted by this longirostrate multi host tick are a contributory factor in the deterioration of hide quality and losses to the leather industry.[3] The other direct damages include losses in milk production and body weight along with increased mortality, whereas the indirect losses are its role as vector, transmitting various economically important hemoprotozoan parasites

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(*Theileria annulata*, *Theileria buffeli* and *Theileria lestoquardi*) to the livestock. In addition, the role of *Hyalomma* species has been established in transmitting the crimean-congo hemorrhagic fever virus in humans.[4] The cost for management of ticks and tick borne diseases in livestock of India has been reported in tune of US$ 498.7 million per annum.[5]

The commonly available chemical acaricides such as organophosphates (OP) and synthetic pyrethroids are widely being used for control of ticks, but their indiscriminate and persistent use along with poorly supervised applications for an extended time period has led to the development of resistance to these acaricides.[6] Reports of OP resistance in ticks are mostly available against one host cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* populations worldwide[7-9] including India.[10-12] There is little information regarding acaricidal resistance in multi-host ticks;[13] however, recent studies from India had shown development of resistant *H. a. anatolicum* populations against commonly used acaricides.[14-18] There are few reports available on malathion resistance from Punjab state and that too against *R.* (*B.*) *microplus* ticks;[6,12] hence, the current study was undertaken to detect malathion resistance in *H. a. anatolicum* collected from Bathinda district, Punjab.

MATERIALS AND METHODS

Collection of ticks

Live engorged *H. a. anatolicum* adult female ticks were collected from sheds of dairy animals comprising of cross bred cattle and buffaloes from Bathinda district, Punjab, in August, 2014. The ticks were collected in plastic vials, closed with muslin cloth to allow air and moisture exchange, brought to the Entomology Laboratory, Department of Veterinary Parasitology, GADVASU, Ludhiana, and utilized for bioassay.

Acaricide

Technical grade malathion (AccuStandard® Inc., USA) was used to prepare the stock solution in methanol. For the bioassay, different concentrations of malathion were prepared in distilled water from the stock solution and tested against *H. a. anatolicum*.

Adult immersion test

Adult immersion test (AIT) was conducted according to the method of Sharma *et al*.[19] with minor modifications. The collected ticks were thoroughly washed with water, dried with paper towels, and randomly divided in groups of 10 each for generation of dose mortality response data against malathion. Briefly, the preweighed engorged female ticks were immersed in various increasing concentrations of malathion (625, 1250, 2500, 5000, and 10,000 ppm) for 2 min and then dried on filter paper before transferring into Petri dishes. After 24 h, individual ticks were transferred to glass tubes covered with muslin cloth, kept in desiccators, and placed in incubator maintained at 28°C ± 1°C and 85% ± 5% RH. The ticks which did not oviposit even after 14 days posttreatment were considered as dead. The control group was treated in a similar manner with distilled water and the following parameters were compared:

1. Mortality: Recorded up to 14 days posttreatment
2. The egg masses laid by the live ticks
3. Reproductive index (RI) = egg mass weight/engorged female weight
4. Percentage inhibition of oviposition (% IO) = ([RI control - RI treated]/RI control × 100).

Dose response data were analyzed by probit method[20] using GraphPad Prism 4 software (San Diego, California, USA). The LC_{50} and LC_{95} values of malathion were determined by applying regression equation analysis to the probit transformed data of mortality.

Resistance diagnosis in field isolates

Resistance factors (RF) against malathion in *H. a. anatolicum* ticks were worked out as per the method of Jyoti *et al*.[12] On the basis of RF, the resistance status was classified as susceptible (RF < 1.4), level I resistant (RF = 1.5–5.0), level II resistant (RF = 5.1–25.0), level III resistant (RF = 25.1–40), and level IV resistant (RF > 40.1) as per Sharma *et al*.[19]

RESULTS

The data on the dose-mortality response of *H. a. anatolicum* ticks collected from Bathinda district, Punjab, against malathion are presented in Table 1. Interestingly, exposure to concentration recommended for field usage (5000 ppm) could achieve only 30% mortality, and even much higher concentration of 10,000 ppm failed to produce cent percent mortality indicating development of resistance against malathion. The regression graph of probit mortality in ticks was plotted against log values of progressively increasing concentrations of malathion [Figure 1]. The dotted lines in the regression curve represented the 95% confidence interval (CI). The slope of mortality (95% CI) was 2.489 ± 0.719 (0.2002 to 4.778), whereas the value of goodness of fit (R^2) was recorded as 0.799. From the regression equation, the lethal concentration values of malathion to kill 50 (LC_{50}) and 95% (LC_{95}) together with their respective 95% CI were recorded as 9099.2 (8378.6−9881.7) and 41,511.3 (35,060.2−49,149.4) ppm, respectively. The analysis of data revealed a RF of 16.60 indicating level II resistance status [Table 2].

The effect of exposure of increasing concentrations of malathion on reproductive parameters of engorged

Table 1: Dose-dependent response of malathion against *Hyalomma anatolicum anatolicum* collected from Bathinda, Punjab

Concentration (ppm)	Weight (mg) (mean±SE)	Mortality (%)	Egg mass weight (mg) (mean±SE)	RI[a] (mean±SE)	% IO[b] (mean±SE)
625	281.1±17.91	0.0	154.5±18.25	0.542±0.04	3.80±7.80
1250	243.1±10.50	0.0	125.9±7.90	0.520±0.02	7.77±5.04
2500	293.5±12.61	0.0	140.0±8.16	0.480±0.02	14.85±4.41
5000	260.1±11.67	30.0	92.5±13.04	0.341±0.04	39.4±7.13
10,000	244.5±18.90	70.0	33.0±3.56	0.189±0.02	66.4±4.17
Control	301.4±14.26	0.0	170.5±11.93	0.564±0.02	0.0

[a]RI=Egg mass weight/live tick weight, [b]% IO: (RI control - RI treated)/RI control×100). RI = Reproductive index, SE = Standard error, % IO = Percentage inhibition of oviposition

Table 2: Slope, LC_{50}, LC_{95}, 95% confidence limit and RF values of malathion against *Hyalomma anatolicum anatolicum*

Variables	Slope±SE (95% CI)	R^2	LC_{50} (ppm) (95% CI)	LC_{95} (ppm) (95% CI)	RF[c] (RL[d])
Mortality	2.489±0.719 (0.2002 to 4.778)	0.799	9099.2 (8378.6-9881.7)	41,511.3 (35,060.2-49,149.4)	16.60 (II)
Egg mass weight	−91.79±25.15 (−171.8 to −11.76)	0.816			
RI[a]	−0.293±0.059 (−0.482 to −0.105)	0.891			
% IO[b]	52.08±10.49 (18.68 to 85.47)	0.891			

[a]RI=Egg mass weight/live tick weight, [b]% IO=(RI control - RI treated)/RI control×100), [c]RF = Resistance factor, [d]RL = Resistance level. % IO = Percentage inhibition of oviposition, RI = Reproductive index, CI = Confidence interval, SE = Standard error

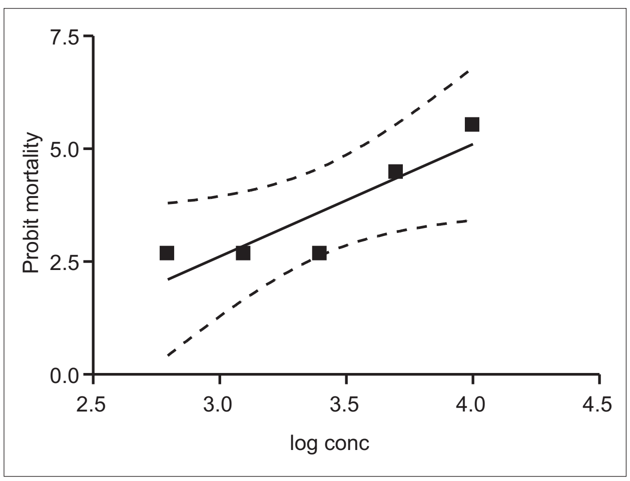

Figure 1: Dose mortality curve of *Hyalomma anatolicum anatolicum* against malathion

H. a. anatolicum ticks viz. egg mass weight, RI, and % IO were studied by AIT [Table 2]. A decrease in the mean egg mass weight of treated ticks was recorded and the slope (95% CI) was −91.79 ± 25.15 (−171.8 to −11.76). A negative dose-dependent slope was recorded because with the increasing concentrations of malathion the survived ticks laid significantly (P = 0.0355) few eggs [Figure 2]. Consequently, the mean RI of treated ticks showed a decreasing dose-dependent response and the slope (95% CI) was −0.293 ± 0.059 (−0.482 to −0.105). Results, thus, indicate that although the increase in concentration of malathion may have not caused cent per cent mortality in ticks, the survived ticks showed a significant decrease (P = 0.0158) in their efficiency to convert their live weight into egg mass [Figure 3]. Furthermore, a dose-dependent significant increase (P = 0.0157) in

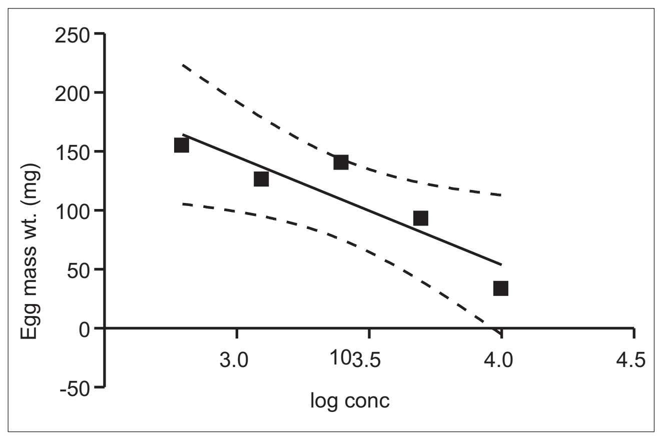

Figure 2: Pattern of egg mass weight of *Hyalomma anatolicum anatolicum* against malathion

the mean % IO of treated ticks along with a positive slope (95% CI) of 52.08 ± 10.49 (18.68–85.47) was recorded [Figure 4]. The above findings indicate presence of significant dose-dependent effect of malathion on the reproductive parameters of *H. a. anatolicum*.

DISCUSSION

In the current study, AIT with a 14 days oviposition protocol and an immersion time of 2 min was used[11,15] for the detection of malathion resistance in *H. a. anatolicum*. The direct mortality was taken into consideration by comparing female ticks that either oviposit or do not on 14 days posttreatment. Although larval packet test (LPT), originally described by Stone and Haydock,[21] has been recommended by FAO as standard bioassay for testing resistance to acaricides in ticks, other tests such as larval

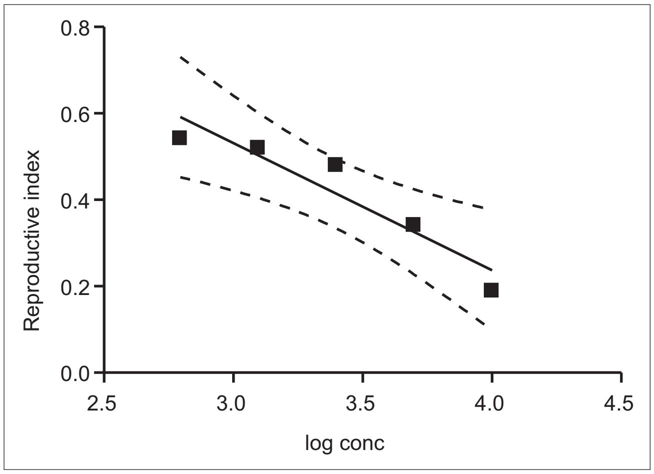

Figure 3: Pattern of reproductive index of *Hyalomma anatolicum anatolicum* against malathion

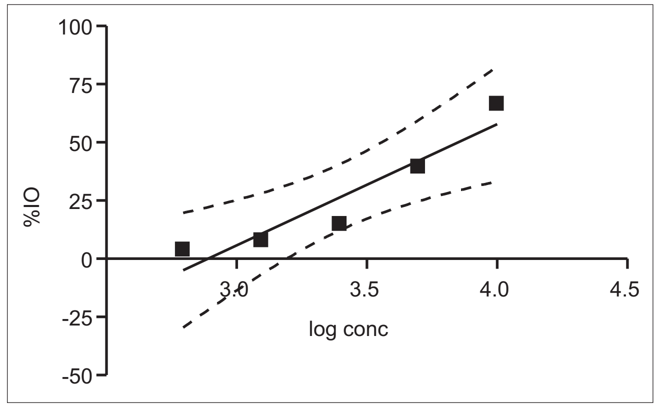

Figure 4: Pattern of percentage inhibition of oviposition of *Hyalomma anatolicum anatolicum* against malathion

immersion test[22] and AIT[23] have also been used. The LPT has not been adopted in all countries as it takes between 5 and 6 weeks to complete and is a laborious test that requires a significant amount of monetary and laboratory resources. On the other hand, AIT can be easily conducted without any special equipment requirement and can be completed within 1 week.[24]

However, in the current study, we got consistent results when egg masses were recorded on 14 days posttreatment as described earlier by Sharma *et al*.[19] Further, AIT has extensively been used worldwide by various workers for estimation of resistance status against various acaricides in ticks.[11,15,17,19,24] For conducting the bioassay, technical grade malathion 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.[22] Use of organic solvent (methanol) facilitates the adsorption of compound over surface area of the target biological materials and also enhances the penetration of active ingredients of acaricide across the exoskeleton.[19] Therefore, the stock solution of malathion was prepared in methanol and working concentrations in distilled water.

As regards Indian scenario, currently, overdependence on the chemical acaricides for tick control in livestock makes the problem of resistance development in these pests inevitable. Further, various factors such as indiscriminate usage, inappropriate dosage, and extended use of any drug for prolonged time periods has probably contributed toward the development of acaricidal resistance.[6] Several reports of OP resistance in *R.* (*B.*) *microplus* ticks from various parts of world[7-9] including India[10-12] are available. Acaricide resistance development in ticks is not universal, and is most widespread and diverse in the one-host cattle tick *R.* (*B.*) *microplus* as compared to multi-host ticks.[13] In multi-host ticks, the resistance develops at a comparatively slower rate as a much lower fraction of the total tick population remains under chemical challenge at any 1 time.[17] In addition, a single generation of multi-host tick may extend over a much longer period of time (up to 3 years) when compared to *Boophilus* species (2–3 months).[25] Recent studies have shown the presence of acaricide resistance against various commonly used acaricides in *H. a. anatolicum* from India.[14-18] However, till date, reports on malathion resistance are only available from one-host tick, *R.* (*B.*) *microplus*.[10,12] Hence, the current study seems to be the pioneer report of malathion resistance in multi-host tick, *H. a. anatolicum*. The results of the current study would be useful in the formulation and implementation of effective tick control strategies in the region.

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DST, New Delhi.

Conflicts of interest

There are no conflicts of interest.

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