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 $2.489 \pm 0.719$ $(2.489 \pm 0.719)$ 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 ($-91.79 \pm 25.15$ $[-171.8 \text{ to } -11.76]$) 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 $-0.293 \pm 0.059$ $(-0.482 \text{ to } -0.105)$. 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, particularly Punjab state. 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. 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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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
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
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.\textsuperscript{(19)} Further, AIT has extensively been used worldwide by various workers for estimation of resistance status against various acaricides in ticks.\textsuperscript{[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.\textsuperscript{(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.\textsuperscript{(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 acaridial resistance.\textsuperscript{(6)} Several reports of OP resistance in R. (B.) microplus ticks from various parts of world\textsuperscript{[7–9]} including India\textsuperscript{[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.\textsuperscript{(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 time.\textsuperscript{(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).\textsuperscript{(25)} Recent studies have shown the presence of acaricide resistance against various commonly used acaricides in H. a. anatolicum from India.\textsuperscript{[14–18]} However, till date, reports on malathion resistance are only available from one-host tick, R. (B.) microplus.\textsuperscript{[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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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Ghosh S, Ray DD, Vanlahmuaka, Das G, Singh NK, Sharma JK, et al. Progress in development of vaccine against Hyalomma anatolicum anatolicum-Indian scenario. Vaccine 2008;26 Suppl 6:G40-7.
2. Singh NK, Rath SS. Epidemiology of ixodid ticks in cattle population of various agro-climatic zones of Punjab, India. Asian Pac J Trop Med 2013;6:947-51.
3. Biswas S. Role of veterinarians in the care and management during harvest of skin in livestock species. In: Proceedings of National Seminar on Leather Industry in Today’s Perspective, Kolkata, India: 2003. p. 62-4.
4. Gordon SW, Linthicum KJ, Moulton JR. Transmission of Crimean-Congo hemorrhagic fever virus in two species of Hyalomma ticks from infected adults to cofeeding immature forms. Am J Trop Med Hyg 1993;48:576-80.
5. Minjauw B, McLeod A. Tick-borne diseases and poverty. The impact of ticks and tick borne diseases on the livelihood of
small scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK, 2003. p. 59-60.

6. FAO. Resistance Management and Integrated Parasite Control in Ruminants. Guidelines. Animal Production and Health Division: FAO; 2004. p. 25-77.

7. Miller RJ, Davey RB, George JE. First report of organophosphate-resistant _Boophilus microplus_ (Acari: Ixodidae) within the United States. _J Med Entomol_ 2005;42:912-7.

8. Mendes MC, Pereira JR, Prado AP. Sensitivity of _Boophilus microplus_ (Acari: Ixodidae) to pyrethroids and organophosphate in farms in the Vale Do Paraiba Region, Sao Paulo, Brazil. _Arq Inst Biol_ 2007;74:81-5.

9. Baffi MA, de Souza GR, de Sousa CS, Ceron CR, Bonetti AM. Esterase enzymes involved in pyrethroid and organophosphate resistance in a Brazilian population of _Rhipicephalus (Boophilus) microplus_ (Acari, Ixodidae). _Mol Biochem Parasitol_ 2008;160:70-3.

10. Rath SS, Kumar S, Joia BS. Resistance to diazinon and malathion in _Boophilus microplus_ (Acari: Ixodidae) populations from Punjab, India. _J Insect Sci_ 2006;19:74-81.

11. Kumar S, Paul S, Sharma AK, Kumar R, Tewari SS, Chaudhuri P, et al. Diazinon resistant status in _Rhipicephalus (Boophilus) microplus_ collected from different agro-climatic regions of India. _Vet Parasitol_ 2011;181:274-81.

12. Jyoti, Singh NK, Singh H, Rath SS. Malathion resistance in _Rhipicephalus (Boophilus) microplus_ from Ludhiana district, Punjab. _J Parasit Dis_ 2014;38:343-6.

13. Wharton RH, Roulston WJ. Resistance of ticks to chemicals. _Annu Rev Entomol_ 1970;15:381-404.

14. Sangwan AK, Chhabra MB, Singh S. Acaricide resistance status of common livestock ticks in Haryana. _Indian Vet J_ 1993;70:20-4.

15. Shyama KP, Kumar S, Sharma AK, Ray DD, Ghosh S. Acaricide resistance status in Indian isolates of _Hyalomma_ anatolicum. _Exp Appl Acarol_ 2012;58:471-81.

16. Singh NK, Jyoti, Rath SS. Detection of acaricidal resistance in _Hyalomma_ anatolicum anatolicum. _Indian Vet J_ 2013;90:17-9.

17. Singh NK, Jyoti, Haque M, Singh H, Rath SS, Ghosh S. A comparative study on cypermethrin resistance in _Rhipicephalus (Boophilus) microplus_ and _Hyalomma anatolicum_ from Punjab (India). _Ticks Tick Borne Dis_ 2014;5:90-4.

18. Singh NK, Jyoti, Vemu B, Nandi A, Singh H, Kumar R, et al. Laboratory assessment of acaricidal activity of _Cymbopogon winterianus, Vitex negundo_ and _Withania somnifera_ extracts against deltamethrin resistant _Hyalomma anatolicum_. _Exp Appl Acarol_ 2014;63:423-30.

19. Sharma AK, Kumar R, Kumar S, Nagar G, Singh NK, Rawat SS, et al. Deltamethrin and cypermethrin resistance status of _Rhipicephalus (Boophilus) microplus_ collected from six agro-climatic regions of India. _Vet Parasitol_ 2012;188:337-45.

20. Finney DJ. _Probit Analysis – A Statistical Treatment of the Response Curve._ Cambridge: Cambridge University Press; 1962. p. 1-318.

21. Stone BF, Haydock P. A method for measuring the acaricide susceptibility of the cattle tick _Boophilus microplus_ (Can.). _Bull Entomol Res_ 1962;53:563-78.

22. Shaw RD. Culture of an organophosphorus-resistant strain of _Boophilus microplus_ (Can.) and an assessment of its resistance spectrum. _Bull Entomol Res_ 1966;56:389-405.

23. Drummond RO, Ernst SE, Trevino JL, Gladney WJ, Graham OH. _Boophilus annulatus_ and _B. microplus_: Laboratory tests of insecticides. _J Econ Entomol_ 1973;66:130-3.

24. Jonsson NN, Miller RJ, Robertson JL. Critical evaluation of the modified-adult immersion test with discriminating dose bioassay for _Boophilus microplus_ using American and Australian isolates. _Vet Parasitol_ 2007;146:307-15.

25. Harley KLS. Studies on the survival of the nonparasitic stages of the cattle tick _Boophilus microplus_ in three climatically dissimilar districts of north Queensland. _Aust J Agri Res_ 1966;17:387-410.