Explor Anim Med Res,
Vol.10, Issue - 1, 2020, p. 50-54

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

DETECTION OF ORGANOPHOSPHATE (MALATHION) RESISTANCE STATUS IN *HYALOMMA ANATOLICUM* POPULATION FROM SRI MUKTSAR SAHIB DISTRICT OF PUNJAB

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Received 26 May 2020, 9 June 2020

ABSTRACT: Resistance status against organophosphate (malathion) in *Hyalomma anatolicum* collected from Sri Muktsar Sahib, Punjab was estimated by adult immersion test. The engorged female ticks were immersed in progressively increasing concentrations of malathion for 2 min and mortality was recorded up to 14 days post treatment. The LC_{50} (95% confidence interval [CI]) and LC_{95} (95% CI) values were determined as 12364.74 (11230.5-13615.1) and 72379.62 (59376.2-88230.7) ppm, respectively with resistance factor (RF) of 28.95 (Level III resistance). The effect of malathion on reproductive parameters of treated adult female ticks *viz*. egg mass weight (EMW), reproductive index (RI) and percentage inhibition of oviposition (%IO) were also studied. The slope of mean EMW (95% CI) was -111.1 ± 18.79 (-170.9 to -51.30) and negative as with the increasing concentrations of malathion the survived ticks laid significantly (p= 0.0097) fewer eggs. The mean RI of treated ticks showed a decreasing trend and there was a significant increase (p= 0.0064) in the mean %IO in treated ticks with increasing drug concentrations.

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

INTRODUCTION

Ticks are important ectoparasites transmitting pathogens adversely affecting animal health and play a vital role in the economy of livestock industry worldwide particularly in tropical and subtropical regions including Indian subcontinent. As per the recent nomenclature, *Hyalomma anatolicum anatolicum* (Kaiser and Hoogstraal 1964) has now been described as *H. anatolicum* (Apanaskevich and Horak 2005). *Hyalomma anatolicum* (Koch 1844) (Acari: Ixodidae), a three-host tick species is widely distributed in India infesting cattle, buffaloes, sheep and goat, and also transmits the parasitic protozoan of genera *Theileria*, including *Theileria annulata, T. buffeli, T. hirci* (Ghosh *et al*. 2007). Among the 10 species of *Hyalomma* reported, *H. anatolicum* is the most prevalent one from India including Punjab state (Singh and Rath 2013). Tick infestation in livestock leads to low-quality hides due to bite marks inflicted by the longirostrate mouthparts of *Hyalomma*, lowered productivity in terms of weight gain and milk yield and increased mortality (Jongejan and Uilenberg 2004). The annual cost of management of ticks and tick-borne diseases in livestock of India is as high as US$ 498.7 million (Minjauw and McLeod 2003). Besides, the role of *Hyalomma* species in transmitting the Crimean-Congo Haemorrhagic Fever (CCHF) virus in human has also been established (Gordon *et al.* 1993).

In India, about 60% of livestock is reared by small and marginal farmers and use of various organophosphate (OP) compounds is very common for the control of livestock pests (Kumar *et al*. 2011). The OP compounds are also used against agricultural pests and for mass eradication of mosquito larvae in their breeding places (ICMR Bulletin 2002). However, its large scale and repeated applications has resulted in development of resistance in ticks, contamination of food products and environmental pollution (Graf *et al*. 2004). There are several reports of resistance development against several

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classes of acaricides in *H. anatolicum* from India (Singh *et al*. 2015, Prerna *et al*. 2017, 2019, Jyoti *et al*. 2019). Although, OP resistance in one host cattle tick, *Rhipicephalus microplus* is widespread with several reports (Kumar *et al*. 2011, Jyoti *et al*. 2014, 2016, Dutta *et al*. 2017), there is only a sporadic report in *H. anatolicum* from Punjab (Jyoti *et al*. 2015). The current study was undertaken to detect OP (malathion) resistance status in *H. anatolicum* collected from Sri Muktsar Sahib district, Punjab.

MATERIALS AND METHODS

Study area

The fully engorged and dropped female ticks (~ 150) were collected from dairy sheds of district Sri Muktsar Sahib (30° 29' N and 74° 31' E), Punjab with average annual rainfall of 384 mm. The cracks, crevices, loose bricks and all other possible tick hiding places in cattle sheds were thoroughly searched. The ticks were collected in vials, closed with muslin cloth to allow air and moisture exchange and brought to the Entomology Laboratory, Department of Veterinary Parasitology, GADVASU, Ludhiana. As per the identification key of Sen and Fletcher (1962) the collected ticks were identified as *Hyalomma anatolicum*.

Acaricide

Technical grade malathion (AccuStandard® Inc. USA; P-060NB-250, Lot No. 041811MT) was used to prepare the stock solution in methanol (organic solvent). Different working concentrations were prepared from the stock solution in distilled water.

Adult immersion test (AIT)

Adult immersion test was conducted according to the method of Sharma *et al*. (2012) with minor modifications. The collected engorged female ticks were cleaned, dried and ticks with uniform weight were randomly divided in 12 groups of ten ticks each for generation of dose mortality response. Briefly, two groups of engorged females of *H. anatolicum* were immersed in each working concentrations of malathion (1250, 2500, 5000, 10000 and 20000 ppm) and also distilled water (control group) for 2 min. The ticks were dried on filter paper, weighed, transferred to glass tubes covered with muslin cloth and 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 following parameters were compared:

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

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

(c) Reproductive Index (RI) = EMW/engorged female weight (EFW).

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

Estimation of resistance status

The dose response data was analysed by probit method (Finney 1962) using GraphPad Prism 4 software. The LC_{50} and LC_{95} (95% confidence interval [CI]) values of malathion was determined by applying regression equation analysis to the probit transformed data of mortality. Resistance factor (RF) was worked out as per the method of Jyoti *et al*. (2015) and on the basis of RF, the resistance status was classified as susceptible (RF < 1.5), level I (1.5 < RF < 5), level II (5 < RF < 25), level III (25 < RF < 40) and level IV (RF > 40) (Sharma *et al*. 2012).

RESULTS AND DISCUSSION

The dose-mortality data of *H. anatolicum* ticks collected from Sri Muktsar Sahib, Punjab are presented

Table 1. Malathion dose response on mortality and reproductive parameters against *H. anatolicum* from Sri Muktsar Sahib, Punjab.

Conc. (ppm)	Weight (mg) (mean ± SE)	Mortality (%)	Egg mass wt. (mg) (mean ± SE)	RI[a] (mean ± SE)	%IO[b] (mean ± SE)
1250	272.8 ± 20.09	0.0	166.4 ± 16.20	0.610 ± 0.03	10.05 ± 5.15
2500	286.0 ± 19.35	10.0	153.3 ± 21.31	0.547 ± 0.06	19.42 ± 9.42
5000	279.4 ± 20.44	20.0	136.6 ± 27.72	0.482 ± 0.08	28.86 ± 12.06
10000	305.5 ± 28.56	50.0	85.0 ± 33.64	0.256 ± 0.09	62.29 ± 13.70
20000	279.9 ± 25.24	60.0	33.2 ± 16.60	0.108 ± 0.04	83.98 ± 7.34
Control	312.6 ± 13.93	0.0	212.5 ± 10.18	0.679 ± 0.006	0.0

[a]Reproductive Index: Egg mass wt./live tick wt.

[b]Percentage Inhibition of oviposition: {(RI control - RI treated)/RI control × 100}

Table 2. Slope, LC_{50}, LC_{95}, 95% confidence interval (CI) and RF values of malathion against *H. anatolicum* from Sri Muktsar Sahib, Punjab.

Variables	Slope ± SE (95% CI)	R^2	LC_{50} (ppm) (95% CI)	LC_{95} (ppm) (95% CI)	RF[c] (RL[d])
Mortality	2.137 ± 0.2424 (1.366 to 2.909)	0.963	12364.74 (11230.5-13615.1)	72379.62 (59376.2-88230.7)	28.95 (III)
Egg mass weight	-111.1 ± 18.79 (-170.9 to -51.30)	0.921			
RI[a]	-0.429 ± 0.063 (-0.630 to -0.229)	0.939			
%IO[b]	63.30 ± 9.242 (33.89 to 92.71)	0.939			

[a]Reproductive Index: Egg mass wt./live tick wt.
[b]Percentage Inhibition of oviposition (%IO): {(RI control - RI treated)/RI control × 100}
[c]RF - Resistance factor; [d]RL - Resistance level

in Table 1. The recommended therapeutic concentration of malathion (5000 ppm) against ticks (Jyoti *et al*. 2014) caused only 20% mortality and even much higher concentrations (up to 20000 ppm) failed to produce 100% mortality, thus indicating development of resistance against malathion in these ticks. The regression graph of probit mortality in ticks plotted against log values of progressively increasing concentrations of malathion is presented in Fig. 1. The dotted lines in the regression curve represented the 95% confidence interval (CI). The slope of mortality (95% CI) was 2.137 ± 0.2424 (1.366 to 2.909) whereas, the value of goodness of fit (R^2) was 0.963. The LC_{50} (95% CI) and LC_{95} (95% CI) values were recorded as 12364.74 (11230.5-13615.1) and 72379.62 (59376.2-88230.7) ppm, respectively and the RF was 28.95 which indicated level III resistance status in the population (Table 2).

A significant effect was observed on the reproductive parameters of engorged *H. anatolicum* female adult ticks *viz*. EMW, RI and %IO (Table 2). Upon exposure with increasing concentrations of malathion a decrease in mean EMW of live treated ticks was recorded. The slope of mean EMW was negative as with the increasing concentrations of malathion the survived ticks laid significantly (p= 0.0097) fewer eggs (Fig. 2). Further, the mean RI of treated ticks also showed a decreasing trend with increasing concentrations of malathion and the slope was also negative. Thus, even though various concentrations of malathion may have not caused mortality in all treated ticks but the survived ticks showed significant decrease (p= 0.0065) in their efficiency to convert their live weight into egg mass (Fig. 3). There was a significant increase (p= 0.0064) in the mean %IO in treated ticks with increase in acaricide concentration and thus a positive slope of dose- %IO response was recorded (Fig. 4).

In the current study, AIT with a 14 days oviposition protocol (Sharma *et al*. 2012) and an immersion time of 2 min was used (Kumar *et al*. 2011). The bioassay uses engorged adult females which are immersed in acaricides and results are based on the difference of oviposition rate between females of treated and control groups. The direct mortality was taken into consideration by comparing females that oviposit or not on 14 days post treatment. The AIT had been used extensively by various workers for estimation of resistance status against OP acaricides in ticks (Kumar *et al*. 2011, Jyoti *et al*. 2015, 2016; Dutta *et al*. 2017). Further, technical grade malathion was selected over commercial formulations for the bioassay as commercial products are prepared with many proprietary ingredients and it is difficult to assess the responses due to active ingredients (Shaw 1966). The methanolic stock solution was used for preparing aqueous working concentrations as organic solvent facilitates adsorption of acaricide through enhanced penetration across the exoskeleton of the ticks (Kumar *et al*. 2011).

The state of Punjab has been one the front runners in white revolution in the country and is 5th in milk production amongst all the Indian states with cattle and buffalo population of 2.47 and 4.02 million, respectively (BAHS 2019). Infestation of ticks, particularly *R. microplus* and *H. anatolicum* are a major problem for the dairy industry of Punjab state because of the availability of environmental conditions conducive for

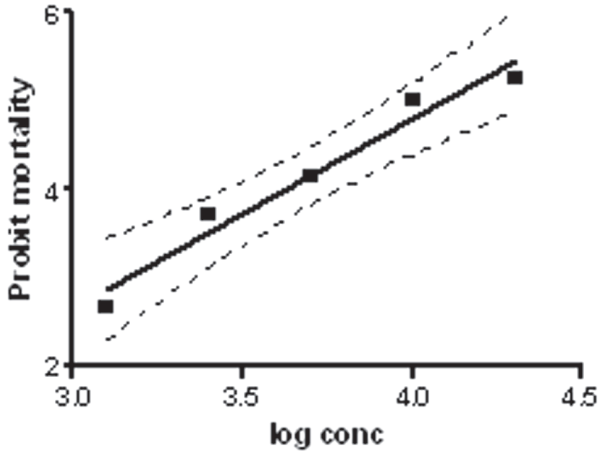

Fig. 1. Dose-mortality curve of *H. anatolicum* against malathion.

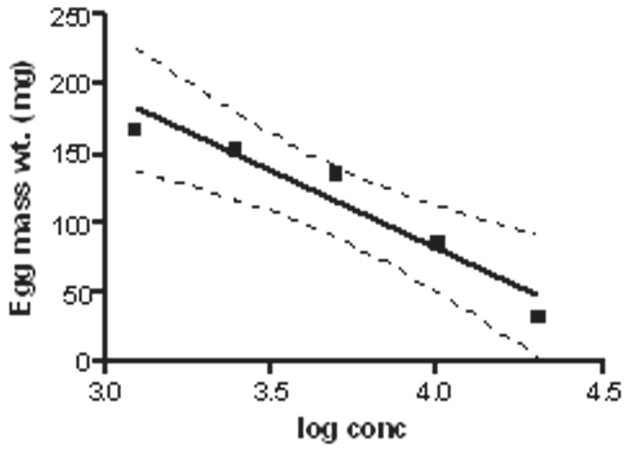

Fig. 2. Dose-egg mass weight curve of *H. anatolicum* against malathion.

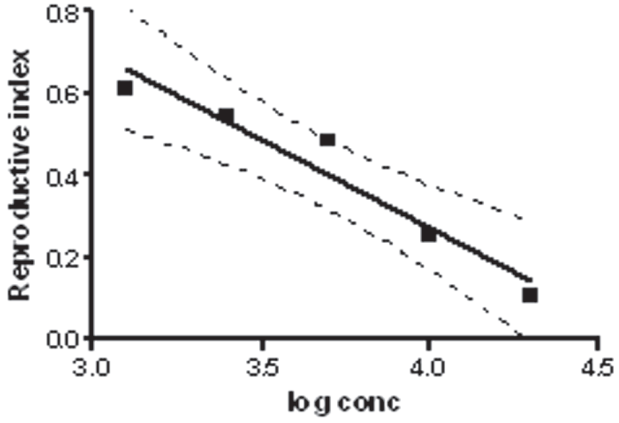

Fig. 3. Dose-reproductive index curve of *H. anatolicum* against malathion.

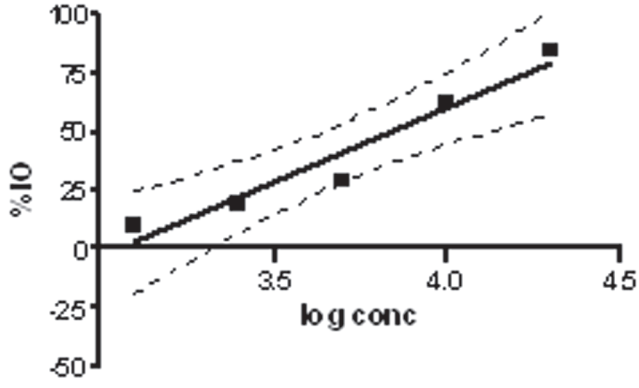

Fig. 4. Dose-percent inhibition of oviposition (%IO) curve of *H. anatolicum* against malathion.

tick survival throughout the most parts of the year and maintenance of susceptible cross bred animals to improve the milk production (Singh and Rath 2013). The most widely used method for the control of ticks is the direct application of acaricides to host animals resulting in the selection of resistant tick populations along with contamination of the environment. Presence of wide spread resistance to the OP compounds in *R. microplus* from various agro-climatic regions of India including Punjab state (Kumar *et al*. 2011, Jyoti *et al*. 2014, 2016; Dutta *et al*. 2017) has been recently reported whereas, level II resistance status against malathion has been reported in *H. anatolicum* from neighboring Bathinda district of Punjab state (Jyoti *et al*. 2015). Therefore, to protect the huge animal population of the state from ticks, monitoring of acaricide resistance is crucial to slow down the process of spreading resistance and to develop region specific strategy for tick control.

ACKNOWLEDGEMENT

The authors are grateful to Department of Science and Technology, New Delhi for funding through Women Scientist Scheme (WOS-A) Project No. SR/WOS-A/LS-493/2011 to the senior author. Sincere thanks are also due to the Director of Research, GADVASU for providing facilities.

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***Cite this article as**: Jyoti, Singh NK, Singh H, Rath SS (2020) Detection of organophosphate (malathion) resistance status in *Hyalomma anatolicum* population from Sri Muktsar Sahib district of Punjab. Explor Anim Med Res 10(1): 50-54.