

Malathion resistance in *Rhipicephalus (Boophilus) microplus* from Ludhiana district, Punjab

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Abstract The resistance status of *Rhipicephalus (Boophilus) microplus* collected from Noorpur Bet village of Ludhiana district, Punjab was evaluated against malathion by Adult Immersion Test. The adult female ticks showed an upward trend in the mortality percentage with increase in drug concentration. The regression graph of probit mortality of ticks plotted against log values of progressively increasing concentrations of malathion was utilized for the determination of slope of mortality (95 % confidence intervals) which was 2.469 ± 0.5744 (0.6413–4.297) whereas, the value of goodness of fit (R^2) was 0.8603. The LC_{50} (95 % CI) and LC_{95} (95 % CI) were recorded as 1875.05 (1725.14–2,038) and 8,654 (7296.8–10263.8) ppm, respectively and the resistance factor was 3.46 (Level I). The slope of egg mass (95 % CI) was -0.1500 ± 0.04071 (-0.2795 to -0.02045) and was negative because with the increasing concentrations of acaricide the ticks died. The reproductive index when plotted against increasing log concentrations of malathion revealed a slope value of -0.414 ± 0.055 . Further, a significant variation ($p = 0.0049$) was recorded in the inhibition of oviposition among the various groups treated with increasing concentrations of malathion.

Keywords Malathion · Punjab · Resistance ·
Rhipicephalus (Boophilus) microplus

Introduction

The cattle tick *Rhipicephalus (Boophilus) microplus* is one of the most important ectoparasites infesting dairy animals of tropics and subtropics particularly Punjab state, India (Haque et al. 2011; Singh et al. 2012a). According to Minjauw and McLeod (2003) the cost of management of ticks and tick borne diseases in livestock of India is as high as US\$ 498.7 million per annum. This parasite causes serious direct and indirect damages to cattle industry. Direct damage includes losses in milk production and body weight along with increased mortality, as well as damage to the leather industry due to hide punctures by this tick. Indirect damages include its role as vector transmitting the causative agents of various economically important haemoprotozoan diseases in dairy animals like babesiosis and anaplasmosis (Jongejan and Uilenberg 1994).

Chemical acaricides particularly synthetic pyrethroids (SP) and organophosphates (OP) are currently the most used method of tick control but the indiscriminate and incessant use with improper concentrations for an extended period of time has probably contributed to the development of resistance to these acaricides (FAO 2004). There are several reports available on acaricidal resistance in *R. (B.) microplus* from various parts of the country (Chaudhary and Naithani 1964; Basu and Haldar 1997; Vatsya and Yadav 2011; Singh et al. 2013) including Punjab state (Singh et al. 2010, 2012b). Recently, large scale resistance to OP compound diazinon (Kumar et al. 2011) and SP particularly cypermethrin and deltamethrin (Sharma et al. 2012) has been experimentally validated in Indian isolates of *R. (B.) microplus*. However, as reports of malathion resistance in *R. (B.) microplus* are scanty particularly from Punjab state (Rath et al. 2006), the current study was undertaken to detect malathion

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resistance in *R. (B.) microplus* collected from Ludhiana district, Punjab.

Materials and methods

Study area

Live engorged *R. (B.) microplus* adult female ticks were collected from sheds of dairy animals comprising cross bred cattle as well as buffaloes from Noorpur Bet village of Ludhiana district, Punjab in October, 2012. Data related to frequency, type and mode of acaricide treatment adopted by the owners and their experiences about the commonly used acaricides efficacy was recorded. The ticks were collected in vials, closed with muslin cloth to allow air and moisture exchange, brought to the Entomology Laboratory, Department of Veterinary Parasitology, GADVASU, Ludhiana, cleaned, labelled and kept at $28 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ relative humidity.

Acaricide

Technical grade malathion (AccuStandard[®] Inc., USA) was used to prepare the stock solution in methanol. For the experimental bioassay, different concentrations of the malathion were prepared in distilled water from the stock solution and tested against *R. (B.) microplus*.

Adult immersion test (AIT)

AIT was conducted according to the method of Sharma et al. (2012) with minor modifications. Briefly, the pre weighed engorged females of *R. (B.) microplus* were immersed in different concentrations of malathion (1,250, 2,500, 5,000, 1,0000 and 20,000 ppm) for 2 min and then dried on filter paper before transferring into the petri dishes. After 24 h, ticks were transferred to the glass tubes covered with muslin cloth and were kept in desiccators kept in BOD incubator maintained at $28 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ 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 and the following parameters were compared:

- Mortality: recorded up to 14 days post treatment
- The egg masses laid by the live ticks.
- Reproductive index (RI) = egg mass wt. (EW)/engorged female wt. (EFW).
- Percentage inhibition of oviposition (IO %) = $((\text{RI control} - \text{RI treated}) / \text{RI control} \times 100)$.

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

LC₅₀ and LC₉₅ value of malathion was determined by applying regression equation analysis to the probit transformed data of mortality.

Resistance diagnosis in field isolates

Resistance factors (RF) were worked out by the quotient between LC₉₅ of field isolates and LC₉₅ of susceptible isolate (half the recommended dose of malathion as discriminating dose is twice the LC₉₅). 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 resistance (RF > 40.1) as per Sharma et al. (2012).

Results and discussion

An increase in the mortality of ticks was recorded along with the increase in concentrations of malathion and 100 % mortality was recorded at 20,000 ppm. It was observed that exposure of ticks to the concentration at which commercial malathion is used (5,000 ppm) could not achieve 100 % mortality and even much higher concentration of 10,000 ppm failed to produce cent percent mortality, thus indicating development of resistance against malathion in these ticks. The regression graph of probit mortality of ticks was plotted against log values of progressively increasing concentrations of malathion (Fig. 1). The dotted lines in the regression curve represented the 95 % confidence limits. The slope of mortality (95 % confidence intervals) was 2.469 ± 0.5744 (0.6413–4.297) whereas, the value of goodness of fit (R^2) was recorded as 0.8603. From the regression equation the various lethal concentration values of malathion were calculated. The LC₅₀ (95 % CI) and LC₉₅ (95 % CI) were recorded as 1875.05 (1725.14–2,038) and 8,654 (7296.8–10263.8) ppm, respectively and the resistance factor was 3.46 which indicated level I resistance status.

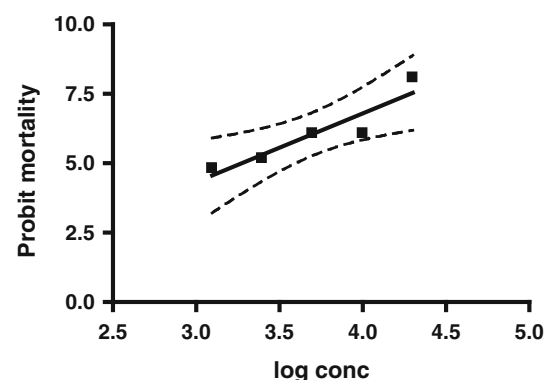


Fig. 1 Dose mortality curve of *R. (B.) microplus* against malathion

The dose response curves for egg masses, reproductive index and IO % of *R. (B.) microplus* were also validated by AIT. The egg mass of ticks treated with different concentrations of malathion decreased with increasing concentrations of drug and the variation was statistically significant ($p = 0.0347$) as shown in Fig. 2. The slope of egg mass (95 % CI) was -0.1500 ± 0.04071 (-0.2795 to -0.02045) and was negative because with the increasing concentrations of acaricide the ticks died. The reproductive index of ticks treated with different concentrations of malathion decreased with increasing concentrations of drug and the slope (95 % CI) was -0.4142 ± 0.05515 (-0.5896 to -0.2387). This indicated that although the increase in concentration of the acaricide may have not caused mortality in ticks but the survived ticks showed significant decrease ($p = 0.0049$) in their efficiency to convert the live weight into egg mass (Fig. 3). Further, there was a significant increase ($p = 0.0049$) in the IO % in ticks with increase in drug concentration and thus a positive slope (95 % CI) of 73.05 ± 9.725 (42.10–104.0) was recorded (Fig. 4).

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 (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 (methanol) 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).

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 viability. Additionally, direct mortality can be taken into consideration by comparing females that oviposit or not (Benavides et al. 1999). In AIT, the results are obtained in 1–2 weeks in contrast with Larval Packet Test which requires 5–6 weeks for

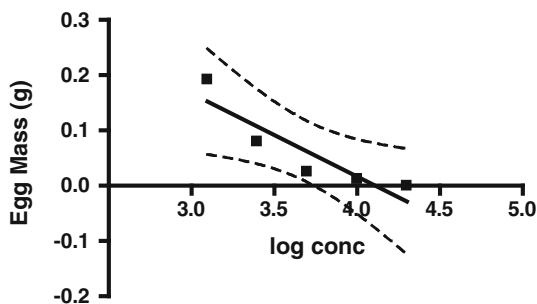


Fig. 2 Pattern of egg masses laid by *R. (B.) microplus* against malathion

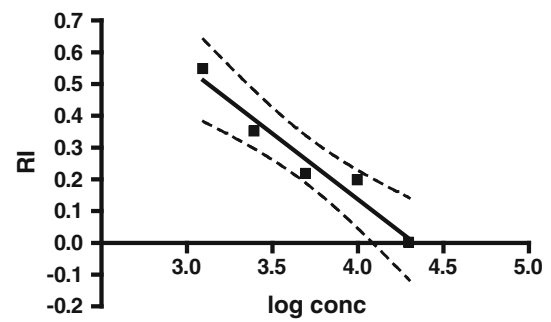


Fig. 3 Pattern of reproductive index of *R. (B.) microplus* against malathion

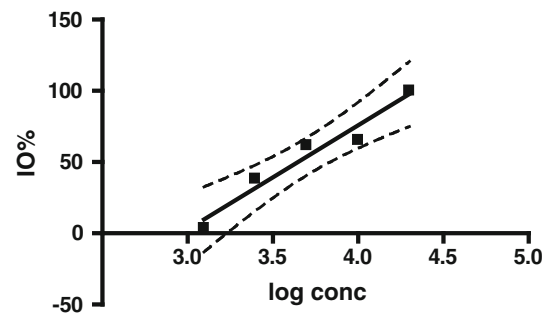


Fig. 4 Pattern of inhibition of oviposition (% IO) of *R. (B.) microplus* against malathion

completion and hence requires less time (Castro-Janer et al. 2009). AIT has been utilized by various workers for estimation of efficacy of various acaricides against *R. (B.) microplus* (Jonsson et al. 2007; Kumar et al. 2011; Sharma et al. 2012; Singh et al. 2012b). Among the various acaricides used in India for the control of ticks in livestock, resistance was first reported against γ BHC in *R. (B.) microplus* (Chaudhary and Naithani 1964; Khan and Srivastava 1977) followed by dieldrin (Sangwan et al. 1993), sevin (Basu and Halder 1997), lindane (Kumar 1999), diazinon (Kumar et al. 2011), SP (Singh et al. 2010, 2012b; Sharma et al. 2012) and amitraz (Singh et al. 2013). Reports of resistance against OP compounds are available against *R. (B.) microplus* ticks from various parts of world (Miller et al. 2005; Mendes et al. 2007; Baffi et al. 2008) but are scanty from India particularly malathion resistance (Rath et al. 2006).

The current study reports the detection of resistance status of *R. (B.) microplus* against malathion and the mechanism involved in the development of this resistance is further being investigated. Several mechanisms have been evolved through which ticks acquire resistance to OP acaricides particularly malathion. OP compounds inhibit the enzyme acetyl cholinesterase (AChE) which plays a key role in nerve impulse transmission by hydrolyzing the neurotransmitter acetylcholine. Further, elevated oxidative detoxification mechanism of cytochrome P450 monooxygenase (Li et al.

2004) and increased GST activity which may also be involved in a metabolic detoxification mechanism of OP compounds (Li et al. 2003) and lead to resistance. The alteration of AChE to an insensitive form in the cattle tick, *R. (B.) microplus* has been demonstrated as an important mechanism for resistance (Baxter and Barker 1998).

The results revealed development of resistance in *R. (B.) microplus* to malathion and addresses an emerging problem of resistance hence, recommendations aiming for effective tick control should be accordingly formulated and implemented. This will lead to judicious use of the drug and would further cause decreased environmental pollution and reduction in the residual effect of acaricides in the animal products like milk and meat.

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