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IN VITRO EFFICACY OF COMMERCIAL PREPARATION OF AMITRAZ IN *HYALOMMA ANATOLICUM* TICKS IN NORTH GUJARAT, INDIA

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ABSTRACT:

Hyalomma anatolicum ticks are multi-host ticks responsible for various direct and indirect losses on animals and is also related to spread of zoonotic disease virus i.e. CCHF virus. Control of ticks is totally dependent on repetitive use of chemical compounds leading to development of resistance or inefficacy of drugs. A study was conducted to assess the efficacy of commercial preparation of Amitraz in *H. anatolicum* ticks of North Gujarat by two step stratified random sampling procedure from areas having complain of treatment failure by using Amitraz. Larval Packet Test (LPT) was conducted using field strain for determination of 50 and 95% lethal concentration of Amitraz. Results obtained from the test showed inefficacy of Amitraz in tick isolates from Vasda tick isolate (LC 50- 18340 ; LC 95- 1769636) and Palanpur tick isolate (LC 50- 8949.6; LC 95- 2640503) which are much higher than market recommended dose rate. The data on field status of efficacy of amitraz from the area with diversified animal genetic resources will be helpful to implement suitable strategy to overcome the process of development of resistance in ticks.

KEY WORD: Amitraz, *Hyalomma anatolicum*, Larval Packet test, Acaricidal activity.

INTRODUCTION:

Ticks are the most competent arthropod having extensive vectorial potential, transmitting a number of pathogenic organisms like protozoans, viruses, rickettsiae, spirochaetes (Ghosh *et al.*, 2007). *Hyalomma anatolicum* is an extensively distributed multi host tick infesting livestock and responsible for transmitting *Theileria annulata*, *T. buffeli* and *T. lestoquardi* (*T. hirci*) in India. These multi host ticks are also responsible for economic losses both directly and indirectly (Singh *et al.*, 2015). According to Geeverghese and Dhanda (1987) tick infestation is mostly profound in areas having dry regions with extremes of temperatures. Banaskantha district of Gujarat state, possess arid climatic zone with hot summers, favourable for propagation of multi-host ticks. The most recurrent method for the control ticks is repeated use of chemical acaricides. In this regard commonly available acaricides like cypermethrin and deltamethrin (synthetic pyrethroids) and amitraz (formamidines) are frequently and indiscriminately used without following proper dose protocols resulting in inefficacy of compounds to ticks and thus failure in control programs due to development of acaricide resistance (Sharma *et al.*, 2013 Singh *et al.*, 2015).

Amitraz is a triazapentadiene compound acting as a non-systemic octopaminergic agonist and has been used for the control of ticks on bovines (Garris and George 1985; Curtis 1985; Kagaruki 1996), canines and undomesticated animals (Pound *et al.*, 2000; Elfassy *et al.*, 2001; Kumar *et al.*, 2001), parasitic mites of honeybee (Floris *et al.*, 2001), and other ectoparasites of livestock (Curtis 1985). In India, amitraz is one of the commonly used acaricides for the control of cattle and dog ticks (Mathivathani *et al.*, 2011). There is a dearth of information on acaricidal resistance in multi-host ticks (Wharton and Roulston, 1970), limited studies have been conducted to detect the resistance status in *H. anatolicum* in India (Sangwan *et al.*, 1993; Sharma *et al.*, 2012; Sharma *et al.*, 2013 and Singh *et al.*, 2015) and in other multi-host ticks elsewhere in the world. Amitraz is a new compound which is being used after the development of resistance in commonly used SPs, therefore is not being evaluated widely for its resistance / susceptible status. However, reports from (Singh *et al.*, 2015) put some light on the resistance susceptible status of amitraz to multi-host tick *H. anatolicum*. The present study was conducted to determine the efficacy of market formulated preparation of amitraz in multi-host tick *H. anatolicum* of North Gujarat.

METHODS:

Study area

Live engorged adult female *Hyalomma anatolicum* ticks were collected from different organized and unorganized farms of various talukas and villages from North Gujarat. Talukas were selected as per

the complaint of local veterinarians from which ticks were collected following two step stratified random sampling procedure. Gujarat State has diverse climatic conditions and northern part of state is situated in tropical and subtropical agroclimatic zone which is favourable for ticks. The farms in these areas are having crossbred cattle accompanied with some local non-descript breeds. Ticks were collected from the vicinity of cattle shed (cracks and crevices) from the body of infected animals without damaging their mouth parts. Ticks were then put into clean vials covered with muslin cloth to allow air and moisture exchange and brought to department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.

Laboratory maintenance

Ticks brought to the laboratory were washed, dried and were then identified (Sen and Fletcher, 1962 and Geevarghese *et al.*, 2000). Five adult engorged ticks were placed in labeled glass vials, which were then placed individually in desiccator in BOD incubator at $28\pm 1^{\circ}\text{C}$ and $85\pm 5\%$ relative humidity for oviposition. A period of about 15 days was required for *H. anatolicum* for laying of eggs. The eggs were allowed to hatch to larvae for 28-35 days under similar conditions of incubation. The 14 day old larvae were then used for the *in vitro* assay.

Acaricides

Serial dilutions of market formulated preparation of amitraz (12.5%); 125ppm, 250ppm, 500ppm, 1000ppm, 2000ppm was prepared using distilled water as diluent.

Larval Packet Test (LPT)

The LPT was conducted as per the guidelines of FAO (1971) with minor modifications. Briefly, 0.6 ml of different concentration of amitraz in distilled water were used to impregnate 3.75 cm by 8.5 cm filter paper rectangles (Whatman filter paper no. 1, W & R Balston Limited). The acaricide dissolved filter paper was then dried by keeping it for 30 min in incubator at 37°C . The rectangles were then folded in half and sealed on the sides with adhesive tapes forming an open-ended packet to place tick larvae. Approximately 100-150 larvae were inserted with the help of fine paint brush and the packet was sealed with adhesive tape. These packets were then placed in a desiccator in BOD incubator maintained at $28\pm 1^{\circ}\text{C}$ and $85\pm 5\%$ RH. The packets were then removed after 24 h, and live and dead larvae were counted for mortality calculation. Control packets impregnated with diluent only were also prepared for each series of concentration to be tested.

Statistical analysis

LC_{50} and LC_{95} concentrations of amitraz against *H. anatolicum* were calculated by plotting regression curve of mortality (probit) against values of acaricide concentrations (log) (Finney, 1971).

The efficacy was assessed by comparing the LC₅₀ and LC₉₅ concentrations with the market formulated dose rate.

RESULTS:

For determination of efficacy against amitraz for *H. anatolicum* dose dependent mortality against different concentration of drugs was calculated. Log concentration of drugs and probit mortality was also determined, to calculate LC₅₀, LC₉₅ regression graphs were plotted between probit mortality and log concentration for determination of slope and R² values for different tick isolates. For the determination of efficacy, % mortality of field isolate was compared with the recommended dose of market formulated drug. Details of slope, LC₅₀ and LC₉₅ are given in Table 1.

All the tick isolates listed in Table 1, showed a higher degree of mortality except for Vasda tick isolate and Palanpur tick isolate. The LC₅₀ and LC₉₅ values for Vasda tick isolates for amitraz were 18340, 1769636 respectively and that for Palanpur tick isolate is 8949.67 and 2640503 respectively. The other tick isolates i.e. of Mahadevpura, Deesa, Vav, Jekada, Shihori, Soneth and Bhadath were having very less LC₅₀ and LC₉₅ values showing high degree of mortality with increase in concentration of drugs. Regression graphs between probit mortality and log concentration of drugs for isolates showing inefficacy to commercial preparation are given in Figs. 1 and 2. Whereas other isolates are showing efficacy against amitraz having high rate of mortality at recommended and higher concentration of drugs.

DISCUSSION:

India, despite of low production per animal, ranks first in milk production, accounting for 18.5% of world production (Press information bureau). Dairy farming is a income source for many farmers. Ever growing human population exerts great pressure on livestock production system. Indigenous breeds are resistant to tick infestation but they are low producers. So to maintain the productivity status efforts were done in past to increase milk production by means of crossbreeding. Increased exotic inheritance led to the development of such animal genetic resource in country which are susceptible to many diseases of tropical climate, amongst which tick borne diseases owes a special concern (Bansal, 2005). The control of tick population in our country is entirely dependent on use of chemical acaricides. These acaricides are directly being applied indiscriminately at repeated times of year without following proper dose protocol. The problem of ticks and tick borne diseases are prevalent in our country due to favourable environmental conditions for survival of ticks and their progeny throughout the year. Amitraz, a formamidine acaricide, plays an important role in the control of the ticks that infest cattle and pet animals. As it has minimal toxicity to cattle or humans with no

meat withholding period, thus making it suitable for use in cattle shortly before slaughter (Jonsson and Hope 2007).

Commercial preparation of amitraz with E.C. 12.5% was used to evaluate acaricidal efficacy against *H. anatolicum* using standard bioassay LPT. Amitraz is a new compound for this area and is used by only few farmers for control of ticks (Singh *et al.*, 2015). Switching over to these drugs is recent and at some places they are being used due to inefficient killing of ticks and their stages by synthetic pyrethroids. In the present study, commercial available acaricide was used to assess the efficacy of the drug. As Haque *et al.* (2014) stated that it would not be possible with the use of analytical grade acaricides as commercial products are prepared with many proprietary ingredients and it is difficult to assess the responses due to individual components of formulations. Efficacy of drug was estimated by comparing the LC₅₀ and LC₉₅ values i.e. lethal concentration to 50% of tick larvae tested and lethal concentration to 95% of tick larvae tested respectively from the market recommended doses for these drugs.

In the present investigation efficacy of amitraz for *H. anatolicum* tick isolates was assessed using LPT. Published data for the LC₅₀ and LC₉₅ values of susceptible tick isolates for determination of resistance factor and resistance level for *H. anatolicum* tick isolates is unavailable. Therefore, the efficacy of this drug was assessed by comparing its dose response mortality and LC 50 and LC 95 values from the market recommended dose rate.

The market recommended dose of amitraz is 250 ppm per litre of water. *H. anatolicum* tick isolates from places Vav, Jekada, Shihori, Soneth, Mahadevpura, Deesa and Bhadath showed a high percentage of mortality in the range of 74.92 - 99.09 % with LC 50 (1.3 -16.06) values interpreting that for these tick isolates, amitraz is effective at its market recommended dose. Whereas, in tick isolates of Vasda and Palanpur, even at higher concentrations of drug then the recommended dose i.e. 500ppm, 1000ppm and 2000ppm tick mortality percentage was very low ($\leq 40\%$) correspondingly, LC 50 (18340 & 8949.6 respectively) and LC 95 (1769636 & 2640503 respectively) values for both the tick isolates were in increasing fashion. From these two results i.e. decrease in % mortality and increase in LC₅₀ and LC 95 values interpretation can be made that the market formulation of amitraz is inefficient to kill the *H. anatolicum* ticks and are either resistant or near to resistance. Resistance at level II against amitraz for *H. anatolicum* from Gujarat state has been reported by Singh *et al.* (2015). For the of RF they used the susceptible isolate from the field that were showing nearly 100% mortality as there is no published data on LC 50 for *H. anatolicum* against amitraz. In the present research work susceptible laboratory maintained inbred line for detection of minimum effective concentration were not available, therefore, R.F. for the field isolates could not being detected for *H.*

anatolicum. Similarly, Ravindran *et al.* (2014) worked on *R. (B.) microplus* to test the efficacy of market formulations of acaricides (cypermethrin and fenvalerate) using adult immersion test and concluded that for both drugs when used *in vivo*, the dose rate should be either same or slightly more for getting the similar results. Actual status of resistance in *H. anatolicum* against amitraz in India has not been worked out due to non-availability of a suitable bioassay technique and the discriminating dose (DD) required for detection of resistance (Kumar *et al.*, 2014). The present study supported the findings of Singh *et al.* (2015) with additional information on efficacy of amitraz from the other tick isolates from North Gujarat, since all these isolates were found resistant to number of SP compounds.

CONCLUSION:

Results of the present study states that commercial formulations of amitraz when tested for its efficacy *in vitro* against *H. anatolicum* ticks using larval packet test showed very low percentage of mortality, and increase in LC50 and LC 95 values in tick isolates of Vasda and Palanpur suggesting that amitraz is ineffective at these two places. Therefore, for the control of ticks at these places integrated measures should be incorporated with switch other acaricides for prevention of further resistance.

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Table 1. Larval Packet Test for *H. anatolicum* against amitraz.

Tick isolate	Slope	R ²	LC50	LC95
Vasda	0.8272 ± 1.584	0.08334	18340	1769636
Mahadevpura	0.4252 ± 0.09332	0.8737	1.65	11820
Deesa	0.5647 ± 0.3062	0.5314	16.06	12950
Vav	0.7906 ± 0.1781	0.8679	1.32	157.57
Jekada	0.5182 ± 0.1118	0.8775	1.28	1871.55
Shihori	0.6312 ± 0.2041	0.7621	2.55	1011.01
Soneth	0.7840 ± 0.1261	0.9280	3.295	407.09
Bhadath	1.372 ± 0.4161	0.7837	9.1	143.01
Palanpur	0.6644 ± 0.1455	0.8741	8949.6	2640503

Fig 1. Dose mortality curve against amitraz of *H. anatolicum* from Vasda and Palanpur.

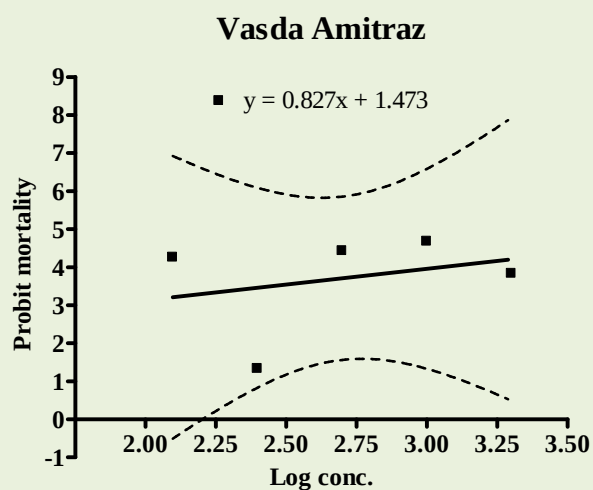


Fig. 1.1

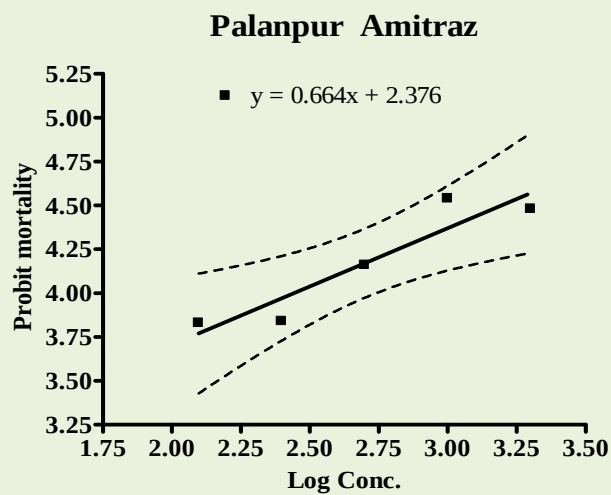


Fig .2