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Detection of Amithraz resistance in *Rhipicephalus* (*Boophilus*) *microplus*, the cattle tick in Telangana, India

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Abstract
In Telangana, the ixodid tick *Rhipicephalus microplus* has developed resistance against synthetic pyrethroids, organophosphates and other acaricides. However, no cases of Amithraz resistance have been reported in this state despite of its heavy use. The modified Drummond Adult Immersion Test was utilized to determine the level of resistance to Amithraz in specimens collected from various areas of Telangana State, India. The regression graphs of probit mortality of *Rhipicephalus microplus* plotted against log values of Amithraz concentration was utilized for the determination of slope, lethal concentration 50%, 95% and resistance factor. The LC_{50} and LC_{95} values of adult immersion test against Amithraz was 116.5, 182.8, 94.56, 64.8, 100.2, 134.7 ppm and 655.1, 1353.5, 553.9, 1326.8, 1463.1, 1288.9 ppm in Rangareddy, Mahaboobnagar, Nizamabad, Warangal, Karimnagar, Khammam district isolates, respectively. Amithraz resistance was evaluated on the basis of a resistance factor (RF), which is the proportion of LC50 field ticks to susceptible ticks. In this study, all 6 isolates from different districts were susceptible to Amithraz showing resistance factor in the range of 0.39 to 1.1. The data generated in this study may be useful to the veterinarians of Telangana state in formulating effective tick control strategies.

Keywords: Cattle, Telangana, *Rhipicephalus microplus* Amithraz, resistance

Introduction
Cattle rearing has always been complimentary to agriculture and it has been a part of Indian households since centuries. Cattle rearing has been the major source of income to the small scale and landless farmers, a majority of whom live below the poverty line. Although India ranks first in the total milk production, the productivity of the cattle is quite low (Pino, 1981) because, of the inferior managemental practices. Various factors including diseases as well as their transmitting agents (ectoparasites) are responsible for deprived health of animals. Of all the ectoparasites, ixodid ticks are the most important blood-feeding ectoparasites of animals and birds (Furman and Loomis, 1984) [6]. These ectoparasites are usually drawn to the host owing to the temperature of the body and their skin texture (Harwood and James, 1979) [7].
Amitraz is an fast working ectoparasiticide that has been used for the control of cattle tick *Rhipicephalus microplus* more than 30 years, even though there are reports of resistance (Chevillon *et al*., 2007; Mendes *et al*., 2013) [1, 13]. Initially Resistance against amithraz has been reported in Australia way back in 1980 against *Boophilus microplus* but the spread of resistance was slow when compared to that of synthetic pyrethroids. However, Many aspects of the Amithraz resistance was less understood. In the present study area of Telangana state also, it appears that the iodide tick, *Rhipicephalus microplus* has developed resistance to a wide variety of insecticides, but, there are no reports in this research area. Hence, the present research was taken up to determine the resistance status of *Rhipicephalus microplus* against Amitraz in severely tick infested cattle belonging to different regions of Telangana state in India for a period of 1 year from Feb 2020 to March 2021.

Materials and Methods
Location and geography of study area
Telangana is a state in the Southern region of India and situated between 16˚-20˚N latitude and 77˚– 82˚ E longitude covering an area of 114,840 km^2 (44,300 sq. m).

Telangana is divided into three agro-climatic zones based on the range of rainfall received, temperature, type and topography of the soils. The details as follows The present study included six districts *viz*., Rangareddy, Mahaboobnagar, Nizamabad, Karimnagar, Khammam and Warangal of Telangana, India where intensive and semi-intensive farms were present.

Collection of ticks

Research study included six districts of Telangana State of India *viz*., Rangareddy, Mahaboobnagar, Nizamabad, Karimnagar, Khammam and Warangal where organised and unorganized dairy farms were present. The blood filled and fallen ticks were collected from cracks, crevices of cattle houses and are placed in separate plastic vials. These vials are closed with muslin cloth for aeration to the ticks. The ticks collected from a different areas was gathered and cleaned properly in distilled water then utilized in bioassay.

Acaricidal preparation

Technical grade amithraz (M/s Accu Standard® Inc, U.S.A.) was utilized for formulating the stock solution in methanol. 1% stock solution was prepared by dissolving 100 mg of Amithraz in 10 ml of methanol. Various concentrations of working solutions were prepared from the stock solution for utilization in the bioassay.

Adult immersion test

The status of the resistance against Amithraz in different district ticks were determined by Adult immersion test according to the method invented by Drummond *et al*, (1973) [2] approved by FAO, (2004). Working concentrations of amithraz in the bioassay included 62.5, 125, 250, 500 and1000 ppm, respectively. Ten adult ticks were used for every replication and each concentration was duplicated four times (n = 4 × 10). The thoroughly washed female ticks were enthralled for 2 min in each concentration of acaricide and dried on filter paper. The Petri dishes with dried ticks were maintained at room temperature for 24hrs. Similarly, the control ticks was treated in 10% working solution of acetone or methanol. Ticks were moved to glass tubes covered with muslin fabric and stored in desiccators then maintained in a BOD incubator at 28 °C and 85% RH. Tick mortality was observed every day for 14 days following treatment. Ticks that failed to lay eggs for up to 14 days and females that produced non-viable eggs were regarded as died.

Estimation of resistance status

Regression curves of Probit mortality were plotted against log values of concentrations (Finney, 1962) using Graph Pad Prism 4 software. The regression curve slope and correlation coefficient were recorded. By dividing each isolate's LC50 by the LC50 of the reference susceptible IVRI-I line, the resistance factor to acaricides for each isolate was calculated. The LC_{50} value of amithraz against susceptive *R*. (*B*.) *microplus* (IVRI-1 line) was 165 ppm (Kumar *et al*, 2014) [11]. Based on the resistance factors, the ticks were classified into susceptible (RF < 1.4), I- (RF > 1.5-5.0), II- (RF > 5.1-25.0), III- (RF > 26-40) and IV- (RF > 41) (Kumar *et al*. 2011) [10].

Results and Discussion

The Adult Immerssion Test indicated a gradually increasing percentage of mortality against Amithraz with increasing concentration of acaricide with highest mortality (95%) at 500 ppm whereas control group showed a mortality of 0.25 percent. Regression plots of Probit lethality of ticks obtained from various isolates plotted on log values of increasing Amithraz concentrations is presented in Fig.1. The effect of various working concentrations of Amithraz on mortality (percent ±SE), slope (95% confidence Limit), goodness of fit (R^2), Lethal Concentration $_{50}$ (95% CL), Lethal Concentration $_{95}$ (95% CL) values, Resistance factor (RF) and level of resistance (RL) in different district isolates against Amithraz were showed in Table 1. The LC_{50} and LC_{95} values of adult immersion test against Amithraz was 116.5, 182.8, 94.56, 64.8, 100.2, 134.7 ppm and 655.1, 1353.5, 553.9, 1326.8, 1463.1, 1288.9 ppm in Rangareddy, Mahaboobnagar, Nizamabad, Warangal, Karimnagar, Khammam district isolates, respectively. The Resistant Factor values ranging from 0.39 to 1.1 revealed a susceptible status when compared to that of susceptible lines.

The results showing, all 6 isolates from different districts were susceptible to Amithraz showing resistance factor in the range of 0.39-1.1. Lower susceptibility of all isolates may be due to fact that the lesser usage of amithraz in the field compared to synthetic pyrethroids led to lower selection pressure and lower resistance frequency could be the reasons for non-appearance of amithraz resistance in the areas under present study. A lower fitness in ticks resistant to Amitraz or a semi dominant mode of inheritance may be attributed to the low prevalence (Kemp *et al*., 2003) [9]. Almost alike results were reported by Dutta *et al* (2017) [3] who reported susceptible and level I resistance to amithraz in Jammu region (India) by adult immersion test (AIT) and hypothesised that, the resistance levels to this acaricides were not alarming. Raynal *et al.* (2020) [14] recorded decreased efficacy of amithraz after repeated usage from 75.05% to 15.8%. Low prevalence of amithraz resistant population were recorded (19.4%) in Mexico due to the continuous use of amitraz from1994 to 2000 in cattle industry (Fragoso and Soberanes, 2001) [5]. Johnson (2007) [8] opined that, at present, Amithraz is the most effective acaricide in Australian cattle despite its heavy use. In India, Kumar *et al.* (2016) [12] reported Amithraz resistance ranging from Level I to III in isolates of northern and eastern states of India. This resistance in north India might be due to the intensive use of Amithraz, with less frequency of application.

Conclusion

The results of the present study revealed a state of effectiveness of Amithraz against cattle tick *Rhipicephalus microplus* in Telangana and may be effective in control of pyrethroid resistant isolates of ticks. Dairy farm owners can manage tick infestations efficiently and cost-effectively by implementing a tick control strategy based on tick resistance data.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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