

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2025): 5.29

IJABR 2025; 9(10): 83-87

www.biochemjournal.com

Received: 02-07-2025

Accepted: 06-08-2025

Manmod Pranita

M.V.Sc. Scholar, Department of Veterinary Parasitology, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra, India

Jumade Pratibha

Assistant Professor, Department of Veterinary Parasitology, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra, India

Kolte SW

Ex Professor & Head, Department of Veterinary Parasitology, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra, India

Corresponding Author:**Jumade Pratibha**

Assistant Professor, Department of Veterinary Parasitology, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra, India

Detection of deltamethrin resistance in *Rhipicephalus microplus* ticks infesting cattle by adult immersion test

Manmod Pranita, Jumade Pratibha and Kolte SW

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i10b.5855>

Abstract

In Nagpur district of Maharashtra State, deltamethrin is the most commonly used acaricide for tick control in livestock. The research aimed to assess deltamethrin resistance in ticks infesting cattle following complaints from livestock owners regarding the diminished efficacy of deltamethrin in several areas of Nagpur district, Maharashtra. This study evaluated the efficacy of deltamethrin against ticks infesting cattle by investigating deltamethrin resistance in ticks from five talukas of Nagpur district of Maharashtra state using *in vitro* method, specifically the adult immersion test (AIT). Commercially available deltamethrin at five distinct concentrations; 25 ppm, 50 ppm, 70 ppm, 105 ppm and 200 ppm were used to assess the resistance through AIT. Based on criteria like adult mortality, reproductive index, oviposition inhibition and egg hatchability of treated female ticks, *Rhipicephalus microplus* female ticks from all five talukas of Nagpur district in Maharashtra were resistant to all the concentrations of deltamethrin compared with control group treated with distilled water with no significant difference. The findings in the current study revealed that *Rhipicephalus microplus* ticks infesting cattle in Nagpur district of Maharashtra state had high levels of deltamethrin resistance.

Keywords: Deltamethrin, resistance, ticks, Nagpur, Maharashtra

Introduction

Ticks are responsible for the production losses of animals by causing tick worry, tick toxicity, sucking of blood, irritancies, damage to hides and weight loss (Furman and Loomis, 1984; Patel *et al.*, 2013) [12, 27]. Tick-borne diseases ranked in fourth position in major infections of livestock (Ghosh *et al.*, 2007) [14]. Economic losses due to tick-transmitted diseases and expenses on control of ticks reduce the cost-effectiveness of animal production. (Singh *et al.*, 2022). Amongst the different tick species *Rhipicephalus microplus* is the most prevalent tick species found in India (Patel *et al.* 2013; Singh and Rath, 2013) [27, 35]. Infestation of cattle tick *Rhipicephalus microplus* (*R. microplus*) is the most predominant tick species in Nagpur district of Maharashtra. (Manmod and Jumade, 2025) [25].

Tick control is done by different methods of which, the use of chemicals is the most commonly used method. Chemical control of ticks comprises the use of various acaricides available in the market. Acaricides include pesticides that kill the members of order Acarina, which includes ticks and mites (Wyk *et al.*, 2016) [41]. Various classes of acaricides used for tick control are natural and synthetic pyrethrins, organochlorides, organophosphate, carbamate, neonicotinoid, formamidine, semicarbazone, phenylpyrazole, and miscellaneous. These acaricides act systemically by direct contact with external applications, or by fumigants or repellents.

As the chemical acaricides plays an efficient role in tick control, the whole world is facing the problem of environmental pollution and acaricidal resistance. Resistance to acaricide is a decreased susceptibility of a parasite to acaricide when it is used at the recommended concentration for which the parasite was previously susceptible. Long-term use of acaricides, improper dilution, wrong application techniques, continuous, indiscriminate use and overdosing have resulted in acaricide resistance development (Abbas *et al.*, 2014; Ravindran *et al.*, 2011) [1, 28]. The development of acaricidal resistance made it difficult to manage and control ticks and tick-borne diseases. (Guerrero *et al.*, 2014) [17]. Acaricidal resistance development seen worldwide as well as in India. Difficulties and expenses involved in developing new acaricide make this topic of research more important (Abbas *et al.*, 2014;

Vudriko *et al.*, 2016; Yessinou *et al.*, 2018, Vatsya, *et al.*, 2011, Shyma *et al.*, 2015, Gaur *et al.*, 2016, Kumar *et al.*, 2017) [1, 40, 42, 38, 33, 13, 24]. By taking all these points in consideration, this research was planned to assess deltamethrin resistance status in *Rhipicephalus microplus* ticks infesting cattle from Nagpur district of Maharashtra.

Materials and Methods

The chemical acaricide from the synthetic pyrethroid group namely; deltamethrin (commercially available) procured from the local market of the area of the study was used in different working concentrations by diluting it with distilled water to evaluate the acaricidal resistance status in *R. microplus* ticks.

Collection of ticks

The study of assessment of deltamethrin resistance in cattle ticks was conducted in five talukas of Nagpur district of Maharashtra state namely; Nagpur, Kamptee, Ramtek, Kalmeshwar and Umred. About ten animals infested with ticks from each of four villages of each district were selected for tick collection. Ticks were collected from different organized and unorganized farms in ventilated, escape-proof sample collection bottles from body of cattle, from the ground, cracks, crevices and under stones of cattle shade. The history of spraying of deltamethrin (yes/no), its frequency, spraying interval, dose, and effectiveness against ticks was asked to the animal owner during tick collection. The ticks were kept cool and transported to the Department of Veterinary Parasitology laboratory for further studies. During the summer season, sterilized sand was kept at the bottom of the bottles on which filter paper and a few drops of water were sprinkled to maintain the moisture level.

In vitro assessment of detection of deltamethrin resistance

The acaricidal activity of deltamethrin against adult ticks was evaluated following the adult immersion test (AIT) as per the protocol described by FAO, (2004) [10] and Drummond *et al.* (1973) [9] with slight modifications. The ticks were weighed and the ticks with approximately similar weights were grouped into six groups each containing six female ticks. The engorged female ticks were immersed in a 1% sodium hypochlorite solution for 1 min, rinsed with sterile distilled water and were dried on sterile filter paper for cuticle antisepsis as per the method described by Angelo *et al.* 2010 and Drummond *et al.* 1973 [9]. Commercially available deltamethrin was diluted in distilled water for preparation of five different working concentrations i.e., 25 ppm, 50 ppm, 70 ppm, 105 ppm and 200 ppm to perform AIT. The stock solution of the control group was prepared using distilled water. The ticks were immersed in 10 ml of concentrations of deltamethrin for 2 minutes with gentle agitation. The ticks in the control group were immersed in distilled water. The ticks were then dried on filter paper and transferred to Petri dishes having Whatman filter paper number no. 1. All the Petri dishes with treated ticks were maintained at room temperature for 24 hours and then each single female tick was transferred to a sterile glass test tube, covered with muslin cloth and kept in a BOD incubator at 28°C and 85±5% RH. (FAO, 2004; Gonçalves, *et al.*, 2007) [10, 16]. The ticks were observed daily for the reproductive

index by oviposition and mortality up to 15 days by observing their pedal reflexes.

The reproductive index and percent inhibition of oviposition were calculated using the formulas (FAO 2004, Gonçalves *et al.*, 2007) [10, 16].

Reproductive Index (RI) = egg mass weight/engorged tick weight

Percentage inhibition of oviposition (%IO) = [(RI control-RI treated)/RI control x 100]

Results and Discussion

In the present study the resistance to the deltamethrin in *R. microplus* ticks from five talukas of Nagpur district of Maharashtra analyzed by AIT revealed the high level of resistance (100%) to deltamethrin at recommended concentration. The deltamethrin concentrations were increased up to 200 ppm still showing the resistance in *R. microplus* ticks. Even at 200 ppm concentration of deltamethrin no mortality of adult female ticks was observed.

After performing AIT, it was observed that *Rhipicephalus microplus* female ticks from all the five talukas of Nagpur district of Maharashtra state were found resistant against deltamethrin by considering the parameters viz; adult mortality, reproductive index, inhibition of oviposition and egg hatchability of female ticks. The data of AIT from Nagpur, Kamptee, Ramtek, Kalmeshwar and Umred is enumerated in Table 1. The dose-dependent response of deltamethrin in engorged *Rhipicephalus microplus* female ticks from all five talukas of Nagpur district of Maharashtra showed the mean percent mortality of engorged female ticks against 25, 50, 70, 105 and 200 ppm of deltamethrin was 0.00 in all the concentrations. The reproductive index (RI) of treated female ticks from all five talukas was reduced at increased concentrations of deltamethrin however RI in the control group was slightly higher as compared to treated female ticks with no more significant difference from respective taluka whereas the calculated values of percent inhibition of oviposition of treated female ticks was increased at higher concentrations of deltamethrin. The percent inhibition of oviposition in all the groups of treated female ticks were non-significant in all the concentrations of deltamethrin. The hatchability of eggs of treated female ticks was observed visually by counting a number of eggs in the treatment group and the hatching of larvae in control group showed no significant difference from all five talukas of Nagpur district of Maharashtra. In the present study, the resistance status of deltamethrin in Nagpur district of Maharashtra was observed up to 200 ppm concentrations even though the recommended concentration of deltamethrin is 25ppm. The ticks found 100 percent resistant to 25 ppm, 50 ppm, 70 ppm, 105 ppm and 200 ppm concentration of deltamethrin which is a big threat for tick control programme. To notice the mortality of ticks up to LC₅₀ and LC₉₅ the concentrations of deltamethrin have to be increased up to a very high level. The increased deltamethrin concentrations in my opinion are against the environmental safety hence the present study was restricted only up to 200 ppm. (Table 1.).

Table 1: Dose dependant evaluation of deltamethrin resistance in engorged *R. microplus* female ticks in various talukas of Nagpur district of Maharashtra state

Concentrations (ppm)	Mean mortality%	Female wt. (mg) N=6	Egg wt. (mg)	Reproductive Index	Inhibition of oviposition	Hatching% (Visual) (N=100)
Taluka-Nagpur						
25	0	427.62±0.006	46.5±0.003	0.108	8.47	91.65
50	0	491.95±0.006	51.95±0.001	0.105	11.01	90.34
70	0	442.07±0.008	45.66±0.002	0.103	12.71	88.98
105	0	447.28±0.003	45.33±0.006	0.101	14.40	86.31
200	0	473.29±0.003	46.33±0.006	0.097	17.79	84.53
Taluka-Kamptee						
25	0	428.33±0.005	45.31±0.001	0.105	11.01	93.75
50	0	423.38±0.006	44.42±0.001	0.104	11.86	92.35
70	0	521.55±0.005	54.20±0.005	0.103	12.71	89.78
105	0	435.73±0.008	44.24±0.002	0.101	14.40	87.98
200	0	466.42±0.003	46.98±0.006	0.100	15.25	84.56
Taluka-Ramtek						
25	0	545.75±0.005	63.98±0.005	0.117	12.03	92.36
50	0	583.98±0.006	68.21±0.006	0.116	13.53	91.87
70	0	567.82±0.004	65.26±0.001	0.114	14.28	90.11
105	0	558.44±0.006	60.38±0.003	0.108	18.79	89.23
200	0	575.62±0.003	61.22±0.001	0.106	20.30	85.77
Taluka-Kalmeshwar						
25	0	323.56±0.005	35.33±0.006	0.109	18.65	92.96
50	0	334.88±0.008	36.5±0.003	0.108	19.40	90.96
70	0	346.71±0.004	36.33±0.006	0.104	22.38	87.53
105	0	387.93±0.001	40.17±0.001	0.103	22.46	84.96
200	0	395.44±0.002	38.87±0.002	0.098	26.86	81.24
Taluka-Umred						
25	0	353.77±0.005	39.11±0.005	0.110	20.28	89.31
50	0	338.89±0.003	36.13±0.001	0.106	23.18	86.91
70	0	363.22±0.006	37.83±0.002	0.104	24.63	85.23
105	0	365.32±0.002	37.28±0.001	0.102	26.08	84.65
200	0	378.56±0.006	37.25±0.003	0.098	28.98	83.41
Control	0	348.32±0.005	48.22±0.001	0.138	0.00	97.32

Coefficient of Variation = 276.700, Treatments found Significant at 1% and 5% level of significance CD (0.01) = 37.958 CD (0.05) = 28.311

Discussion

The presence of deltamethrin resistance was reported in *R. microplus* ticks from many countries including India. High level of resistance against deltamethrin in *R. microplus* ticks was reported by, Singh *et al.* (2010) [36] from Punjab and Sharma *et al.* (2012) [32] from six agro-climatic regions of India. An elevated level of resistance against deltamethrin in *R. microplus* was reported worldwide as well as in India however the low level of resistance against deltamethrin in *R. microplus* was reported by Jyothimol *et al.* (2014) [20] from Kerala, Gaur *et al.* (2016) [13] and Gupta *et al.* (2023) [19] from Haryana and Rajasthan (Tohana tick isolates), Chigure *et al.* (2018) [7] from Uttar Pradesh, Krishnamurthy *et al.* (2019) [22] from Karnataka. Dadas (2015) [8]. Bharkad (2019) [5] and Khating (2023) [21] reported a higher level of resistance against deltamethrin in *R. microplus* ticks from different regions of Maharashtra after performing different bioassays. Whereas, Gaur *et al.* (2016) [13] from Haryana and Rajasthan (Agorha isolates), Gupta *et al.* (2022) [18] from Himalayas of India also reported the susceptibility of *R. microplus* ticks against deltamethrin. In India Ahanger *et al.* (2015) [2] from Jammu and Kashmir reported level I-IV resistance with LC₅₀ at 25.4-3868.8 ppm. Level I-II resistance of deltamethrin with LC₅₀ was reported by Gosh *et al.* (2015) [15] from Bihar at 27.6-467.1ppm, Shyma *et al.* (2015) [33] from Gujrat at 60.05-241.9 ppm, Sagar *et al.* (2020) [31] from Madhya Pradesh at 45.7-1829 ppm, Fular *et al.* (2021) [11] from Uttarakhand at 122.0-390.4 ppm. Similarly Gupta *et al.* (2022) [18] from Himalayas determined

level II-III resistance at 12.6-679.5 ppm after performing AIT. The reports from other countries revealed the presence of resistance against deltamethrin in *Rhipicephalus microplus* ticks reported by Rodriguez *et al.* (2006) [29] from Mexico, Chevillon *et al.* (2007) [6] from New Caledonia, Vudriko *et al.* (2016) [40] from Uganda, Bandara and Karunarathe. (2017) [4] from Sri Lanka, Valsoni *et al.* (2020) [37] from Brazil and Villar *et al.* (2020) [39] from Colombia by performing AIT. Amongst different tick control methods use of chemical acaricides is widely used, hence there is always the risk of developing resistance (Sagar *et al.* 2020) [31]. In synthetic pyrethroid preferably deltamethrin is mostly used by Indian people (Kumar *et al.* 2020). Repeated applications of deltamethrin for control of ticks has resulted in selection of resistant tick populations in almost all tropical and sub-tropical countries (Mendes *et al.*, 2007; Rodríguez-Vivas *et al.*, 2014) [26, 30].

Conclusion

In the present study, it was observed that the commonly used deltamethrin for tick control programme in Nagpur district of Maharashtra developed resistance in *R. microplus* ticks infesting cattle. Also the increased deltamethrin concentrations beyond the recommended levels may be hazardous to human and animal health, nature creatures and may be responsible for environmental pollution so the studies may be conducted to incorporate biological control agents as an alternative to synthetic pyrethroids. Extension education and awareness amongst the farmers should be

implemented to avoid haphazard and indiscriminate use of deltamethrin to overcome the problem of acaricidal resistance.

Acknowledgments

The authors are thankful to Department of Veterinary Parasitology, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur (M.S.) for providing facilities.

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