Asian Journal of Research in Animal and Veterinary Sciences

Volume 6, Issue 3, Page 194-200, 2023; Article no.AJRAVS.99295

To Study the Effectiveness of the Organic Ectoparasiticide "GochidGo" against Cattle Tick Infection in Maharashtra, India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/99295

Original Research Article

Received: 02/03/2023
Accepted: 04/05/2023
Published: 16/05/2023

ABSTRACT

Ticks cause great economic losses to livestock, and tick-borne diseases are one of the emerging threats to livestock and public health in India. The chemical ectoparasiticides are being used to control these ectoparasites, but their residues are reported in milk and meat. Hence, it is important to find effective and eco-friendly control measures for ticks. The present study aimed to determine the efficacy of botanical acaricides against ticks with respect to chemical ectoparasiticides in the Kolhapur district of Maharashtra, India. This study was conducted on the Holstein Frisian breed of cattle. Twenty-four cows from two different herds were selected for the trial, and the selected cows were quantitatively assessed for tick infestation. Four different doses of botanical ectoparasiticide, 'GochidGo', along with conventional chemical ectoparasiticides containing Amitraz 12.5% EC formulation, were taken for the trial. The cow sheds of both locations were treated with GochidGo and Amitraz 12.5% EC formulation at a dose of 7ml/litre of water. Tick mortality was counted in both

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cases. GochidGo treatment had the highest mortality at the dose of 5ml/litre of water, which was at par with Amitraz 12.5% EC formulation. The GochidGo formulation, however, had taken a substantially ($p<0.05$) longer period for ticks re-appearance, demonstrated its long-lasting effects and proved that it is safe for humans and animals.

Keywords: Ectoparasiticide; ticks; Gochidgo; Amitraz; cattle; bio-efficacy.

1. INTRODUCTION

Worldwide, ticks are the second largest vector of human diseases after mosquitoes. Ticks are important animal ectoparasites and their control is essential if livestock production is to meet the world's demand for animal protein. Ticks infect, paralyze and cause physical injury to animals which transmit diseases to livestocks. Global economic losses from tick infestation have been estimated to be between $14,000 and $18,000 million per year, with India accounting for approximately $498.7 million [1]. The successful survival of ticks depends on optimal temperature and humidity, which are the main components of the microclimate in their habitats, and the availability of suitable tick hosts in an urban location suitable for tick life [2]. A tick bite causes direct injury to the animal along with blood loss due to the tick feeding [3]. Ticks are carriers of animal pathogens, causing a large number of diseases by sucking blood [4]. The organisms like *Anaplasma marginale* causes tick fever, are primarily responsible for cattle morbidity in Australia, USA, China and other countries [5]. Several methods are used to treat ticks and tick-borne diseases. In most cases, chemical acaricides are used to control ticks. They kill ticks, but they harm the animals and the environment. Chemical acaricides cause residues in milk, meat, and also promote the emergence of a resistant strain of ticks. Developing a new acaricide is a long and expensive process. Certain herbal mixtures have 70% efficacy for tick control [6]. A wide range of acaricides, including organophosphates, chlorinated hydrocarbons, carbamates, and synthetic pyrethroids are commonly used to treat ticks. The effectiveness of acaricides depends on the quality and quantity of the active substance. Arsenic was the first effective method for controlling ticks and tick-borne diseases and was used in many parts of the world before chemical resistance was a problem [7]. Acaricide based on chlorinated hydrocarbons and organophosphates was used in the past in many countries of the world, but it causes acute toxicity in livestock and mammals and has developed problems of resistance. According to Anand et al. (2021) there was development of deltamethrin resistance in certain species of ticks in Kerala [8].

Various application methods are used to apply chemicals to surfaces, such as dipping, spraying, watering, etc. Direct application of acaricides to animals is the most popular method of tick control on livestock [3]. Under ideal circumstances and with proper equipment handling, applying acaricides to tick-infested cattle by dipping or spraying can be equally effective without harming the animals or diluting the product [7]. Hundreds of potential acaricides were evaluated for dipping, including a range of tickicides such cottonseed oil, fish oil, crude petroleum, kerosene, creosote, tobacco extract, soap and a mixture of sulphur and kerosene [9,10].

The present study was undertaken to evaluate the tick control effect of botanical acaricides' GochidGo' against chemical alternative products available in the market. A bio-efficacy study of GochidGo product containing *Cinnamomum* extract, *Allium sativum* extract, *Azadirachta indica* extract, *Lantana camera* extract and *Cymbopogon* extract was conducted in the Kolhapur district of Maharashtra on cattle of the Holstein Frisian breed.

2. MATERIALS AND METHODS

2.1 Study Location

The study was conducted at Bhatanwadi village, located in the Karveer tahsil of the Kolhapur district in western Maharashtra. High rainfall along with a long winter season has been recorded in the district. Kolhapur district has a four-month rainy season starting from June to September; a four-month winter season from Oct to Jan; and a four-month summer season from Feb to May. The average annual rainfall of the Kolhapur district is 1200 mm. The average annual temperature ranges from 25 to 30 degrees Celsius.The villages were easily accessible and had large herds of cattle that had not been dipped for the past year. The study was conducted during the dry season (Jan to March).

Tick infection was relatively high as compared to the rainy season. The major economic losses incurred by farmers due to tick infestations and tick-borne diseases included high morbidity and mortalities, as well as reduced production and reproduction performance in cattle.

2.2 Study Details

The study animals were the Holstein Frisian breed of cattle, which were managed properly. Two herds of Holstein Frisian cows were selected for the study. Twenty-four cows from both herds were selected for the trial. The selection criteria were the severity of cattle infestation in the herds, the owner's willingness to try a new product, and whether the animal had many visible ticks on its skin. The age of the heifer was 1-year minimum, with an average weight of 400 to 500 kg per heifer. The ticks were counted on the selected animal before and after the trial. Every animal was examined for the presence of ticks on the head, neck, belly, ears, back, legs, perineum, and tail. Preliminary identification of ticks (based on body morphology and color) was done while the ticks were still attached to the animal body. Secondary observation of ticks for further confirmation is also done under a stereo zoom microscope with the aid of identification [11,12].

Four different doses of GochidGo was taken for the trial and one market product containing Amitraz 12.5% EC, were taken for comparative assessment. The cow sheds of both locations were treated with GochidGo as well as Amitraz 12.5% EC at a dose of 7ml/litre of water. Along with the anti-tick effects of both the ectoparasiticides, their antimicrobial activity has also been studied by disc diffusion method of Balouiri et.al (2016) against the three bacterial isolates of tick infected cattles because microbial tick-borne diseases affect the productivity of livestock animals and cause a significant adverse impact on the animals [13]. The CFU count of all three bacterial isolates were adjusted 1-2 x 10^{-8} CFU/ml and 20 µL of each diluted ectoparasiticide (1ml, 2.5 ml and 5 ml per litre of water) was impregnated into sterile, blank discs 6 mm in diameter. All disc were fully dried before the application on bacterial lawn. Distilled water used as a negative control and vancomycin antibiotic used as a positive control. The antimicrobial activity was evaluated be measuring the diameter of inhibition zone around the disc. Similarly animal skin irritation of both the ectoparasiticides also studied as per the OECD Guidelines 404 “Acute dermal irritation/corrosion” [14]. Formaldehyde 0.8% w/v was used as a standard irritant and only placebo patch was used in the control treatment whereas diluted ectoparasiticides GochidGo and Amitraz 12.5% EC were applied at the dose of 50 µL per transdermal patch (1ml, 2.5 ml and 5 ml per litre of water) in the treated animals. All the formulation were applied under 10 x 10 cm patch to one intact site per animal and wrapped with dressing. After 4 hrs transdermal patches were removed and observed the skin dermal reactions i.e erythema and edema. Tick recurrence have also been investigated. Following treatment was taken for the efficacy trial.

2.2.1 Treatment details

T1: GochidGo 1 ml/litre (2.5 litre water/animal)
T2: GochidGo 2.5 ml/litre (2.5 litre water/animal)
T3: GochidGo 5 ml/litre (2.5 litre water/animal)
T4: GochidGo 7.5 ml/litre (2.5 litre water/animal)
T5: Amitraz12.5% EC 1 ml/litre (2.5 litre water/animal)
T6: Amitraz 12.5% EC 2.5 ml/litre (2.5 litre water/animal)
T7: Amitraz 12.5% EC 5 ml/litre (2.5 litre water/animal)
T8: Control (Water spray)

2.3 Data Collection and Statistical Analysis

Experiment was performed in triplicates. Average ticks per animal were calculated before treatment and after treatment up to 72 hours. Mean and standard deviation were calculated for triplicate. The standard deviation is represented as margin of error in the graph at a confidence interval of 95%. Microsoft Excel (2019) and online resource; GraphPad Quickcalcs were used for statistical analysis. Total dead ticks were calculated by subtracting average ticks per animal before treatment from after treatment survived ticks’ counts [15].

3. RESULTS AND DISCUSSION

With the help of a portable magnifying lens, adults and matured ticks on the animal were preliminary identified (based on body morphology and colour) [11,12]. A variety of tick species were found on the selected animals. The average number of ticks per animal ranged from 45 to 56. Tick mortality was counted after 72 hours of application in both cases. The data was statistically analysed and the standard deviation was calculated using an average of three

replications. The highest mortality was recorded in treatment T3, which was at par with treatment T7 and substantially superior to control treatment T8. In the case of market check Amitraz 12.5% EC formulation, the highest mortality of ticks was recorded in the treatment T7, which was 91% after 72 hrs. of application, details presented in Fig. 1. For GochidGo, one additional dose of 7.5 ml per litre (Treatment T4) was also tested and gave 100% tick control after 72 hrs of application. Reoccurrence of the ticks was observed after 60 days in treatment T3 and after 72 days in treatment T4, which was superior to treatment T7 (Amitraz 12.5% EC, 5 ml per litre of water) where ticks reappeared after 21 days.

In preliminary trials, from the results of disc diffusion screening GochidGo formulation clearly shown antibacterial property against different isolates of tick infected cattle skin where as Amitraz 12.5% EC formulation did not shown any antibacterial effects and their details are presented in Table 1, but there are a number of factors that could influence the results of the disc diffusion assay. Firstly, the diameter of the zone is affected by the rate of diffusion of the antimicrobial compound [16,17] and thus may not exactly represent the potency of the formulation antimicrobial activity. Another important factor is the standardization of the inoculum size. This inoculum size is important to ensure uniform lawn growth as a smaller inoculum size (thin bacterial lawn) may produce falsely large inhibition zones while a bigger inoculum size (thick bacterial lawn) may produce falsely smaller zones.

In the case of skin irritation of the animal, Amitraz 12.5% EC at the dose of 2.5 ml and 5 ml per litre of water shown skin irritation in all the animals, erythema and edema was observed in treatment of Amitraz whereas GochidGo treatments did not shown skin irritation or clinical sign of any irritation in any of the treatments. Erythema is redness of the skin or mucous membranes, caused by hyperemia of superficial capillaries [18]. Edema means swelling caused by fluid in body's tissue. Based on the analysis of all the available parameters studied, it can be inferred that GochidGo was tolerated in experimental cattles and there were no dermal irritation in animals. Presence of flies after spray and odour in both the treatment of ectoparasiticides were also recorded. Amitraz 12.5% EC formulation had a irritant odour, whereas GochidGo had a pleasant lemongrass odour and no flies were seen after spraying of GochidGo.

The favourable climatic conditions like temperature, suitable host, and cool and moist weather of western Maharashtra support the growth of ticks, but it is also observed that in a dry climate, tick incidence is also higher. Improper tick control measures and poor husbandry practices are also responsible factors for tick infestation. Feeding a large number of ticks causes weight loss and anaemia in the animal. Apart from causing irritation or anaemia in cases of heavy infestations, ticks can cause severe dermatitis [4]. These parasites have an immediate impact on cattle in terms of weight increase and milk production [19,20]. Tick paralysis can happen at any time if the weather is warm and humid, although it is more likely in the late winter and early spring when the adult ticks are active [21].

Lack of proper tick control measured in an infested area, unawareness regarding the impact of ticks, lack of adequate veterinary infrastructure, and use of routine chemical acaricides are the factors responsible for high tick loads. Severe incidences of ticks reduce the productivity of an animal as well as increase the chances of animal mortality. Severe tick infections decrease an animal's productivity and increase the possibility of animal death. According to Swai et al (2005) tick-borne diseases were the main cause of low productivity and cattle mortality [22]. However, some authors [23,24,25] believe that animals not treated with acaricides achieve endemic stability against tick-borne diseases in due course of time. In addition, in the absence of routine use of acaricides, morbidities have increased; reduced production, as well as reproduction performance of adult cattle, have also been reported in different places [20,26,27].

Therefore, the present study demonstrated immediate, safe, and long-lasting tick control in an eco-friendly way. The frequent use of chemical acaricides causes major cattle losses through death and loss of productivity, which were reported by farmers. GochidGo showed that it has high acaricidal effects as it has given almost 97 to 100% tick control and the reoccurrence period of ticks has been substantially increased, which proved its durability. GochidGo has contact and a systemic mode of action. It acts as a stomach poison in ticks, disrupting cellular metabolism. GochidGo is extremely fast-acting and causes an immediate knockdown paralysis in ticks.

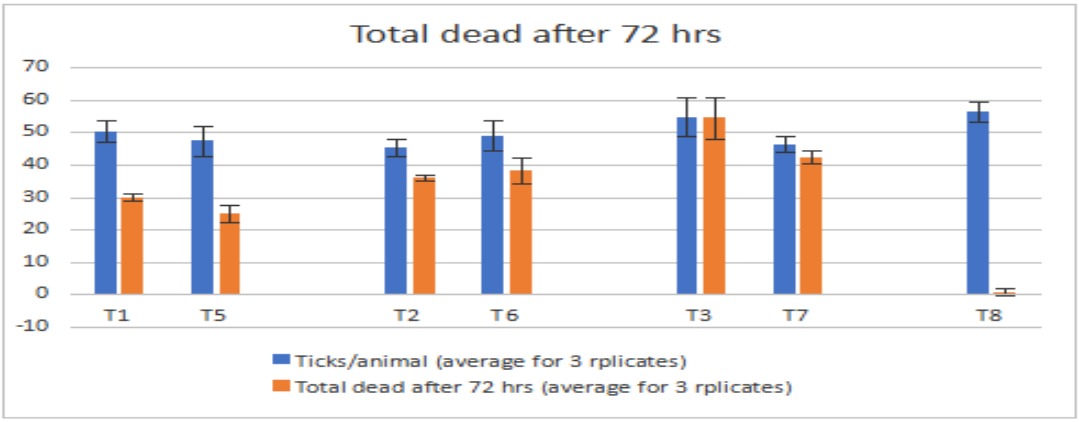

Fig. 1. Effect of GochidGo and Amitraz application on ticks

Table 1. Antimicrobial activity of GochidGo and Amitraz 12.5% EC formulation against bacterial isolates of tick infected cattles

Sr. No.	Formulation Details and dose (20 µL per disc)	Zone of inhibition (mm) against tick infected cattle isolates (Mean of 3 replications)		
		Bacterial Isolate No. 1	Bacterial Isolate No.2	Bacterial Isolate No.3
1	GochidGo 1ml	2.33	2	1
2	GochidGo 2.5 ml	3.33	3.66	2.33
3	GochidGo 5ml	5	4.66	2
4	Amitraz 12.5% EC 1ml	-	-	-
5	Amitraz 12.5% EC 2.5 ml	-	-	-
6	Amitraz 12.5% EC 5ml	-	-	-
7	Vancomycin Antibiotic	8.33	10.66	9
8	Distilled Water	-	-	-

4. CONCLUSION

The results revealed that, the herbal ectoparasiticide GochidGo has outperformed chemical formulations. Ticks were effectively controlled in cattle treated with GochidGo for up to 60 to 72 days. In the case of the Amitraz 12.5% EC formulation, the reappearance of ticks was reported within 21 days after application, i.e., GochidGo gave three-fold longer control of ticks. The GochidGo formulation also acts as a fly repellent as no flies were seen in all the treatments of GochidGo. A irritant odour has been reported after the application of the Amitraz 12.5% EC formulation whereas GochidGo had a pleasant lemongrass odour with no sign of irritation to the animals and operators. The antimicrobial effects of the GochidGo formulation need to be studied further against different disease causing microbes of cattles.

ACKNOWLEDGEMENT

The authors express sincere thanks to Green Vision Life Sciences Pvt. Ltd. for the help rendered and the facilities provided for conducting the study. The authors are also thankful to the farmers of Bhatanwadi village in the Kolhapur District of Maharashtra for allowing the trials on their cattle's.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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Peer-review history:
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